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KUMTA et al.(10) **Pub. No.: US 2023/0381382 A1**(43) **Pub. Date: Nov. 30, 2023**(54) **DEGRADABLE MAGNESIUM-BASED
IMPLANT DEVICES FOR BONE FIXATION**(60) Provisional application No. 62/208,044, filed on Aug.
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C22C 23/04 (2006.01)
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CPC *A61L 31/148* (2013.01); *A61L 31/022*
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2430/02 (2013.01)(73) Assignee: **UNIVERSITY OF PITTSBURGH -
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(US)(57) **ABSTRACT**(21) Appl. No.: **18/204,476**(22) Filed: **Jun. 1, 2023****Related U.S. Application Data**(63) Continuation of application No. 15/750,219, filed on
Feb. 5, 2018, now Pat. No. 11,696,976, filed as
application No. PCT/US2016/048035 on Aug. 22,
2016.

The invention relates to biodegradable, magnesium alloys, compositions and composites, methods for their preparation and applications for their use as implantable medical devices in load-bearing conditions. The magnesium alloys are composed of alloying elements selected from yttrium, calcium, zirconium, zinc, and strontium, with the remainder being magnesium and impurities arising due to production, and preparation of the alloy by melting together the elements and casting the resulting melted mixture. In certain embodiments, the methods of preparation include solution treatment and hot extrusion.

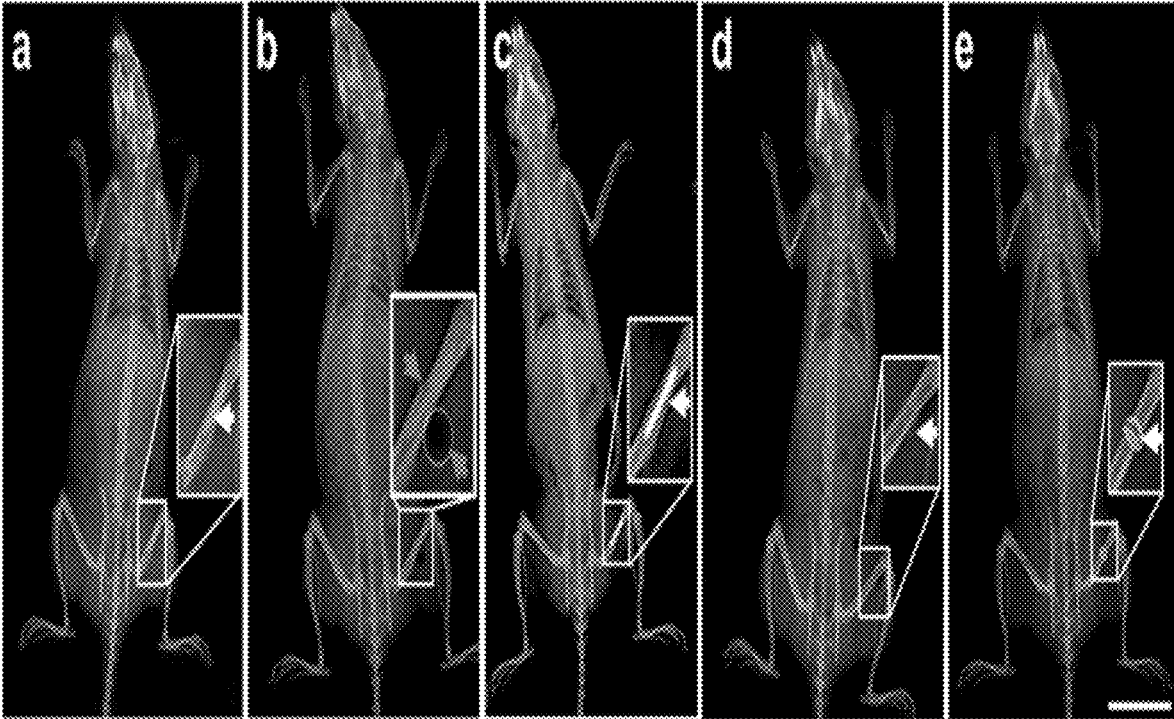


FIGURE 1

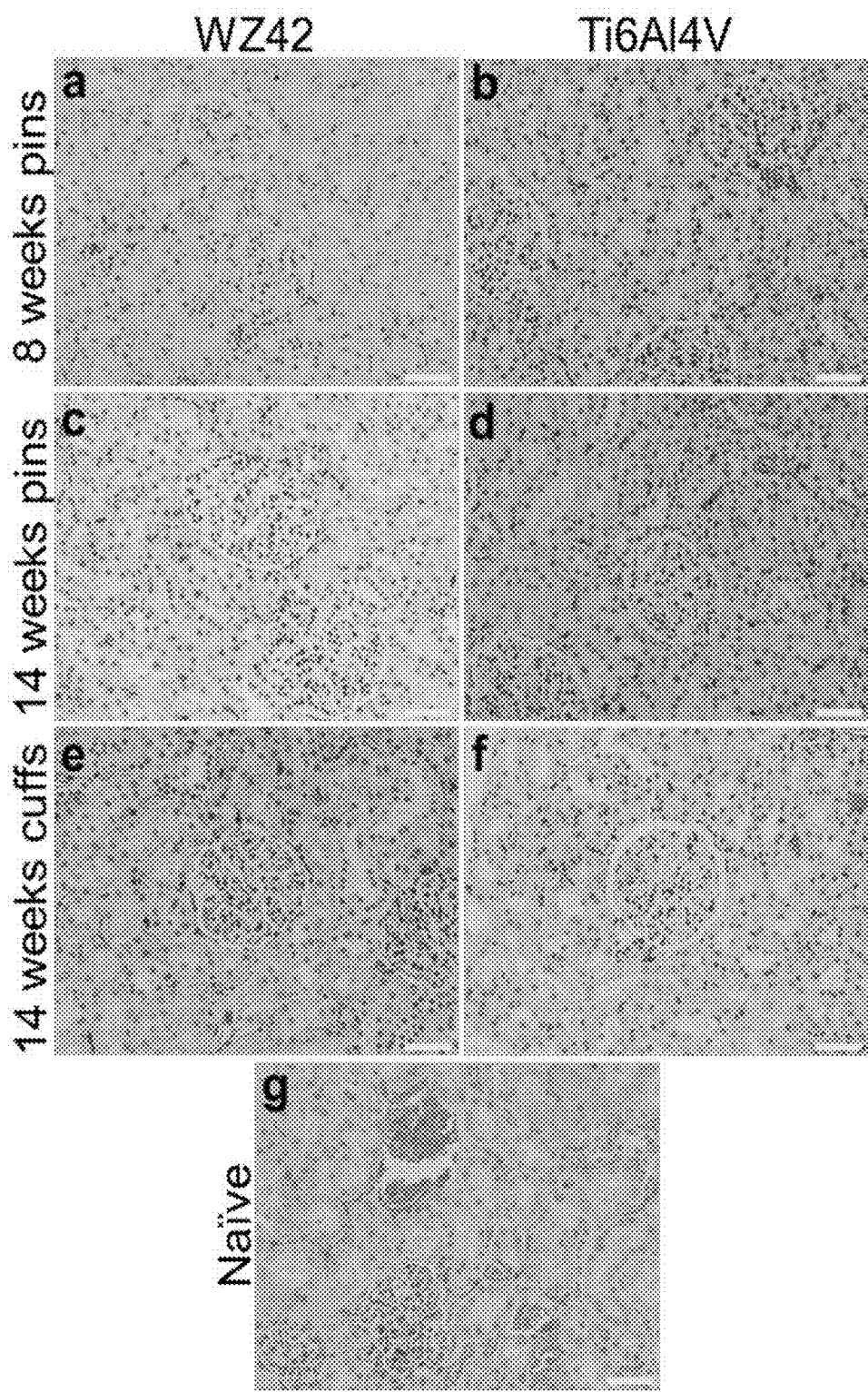


FIGURE 2

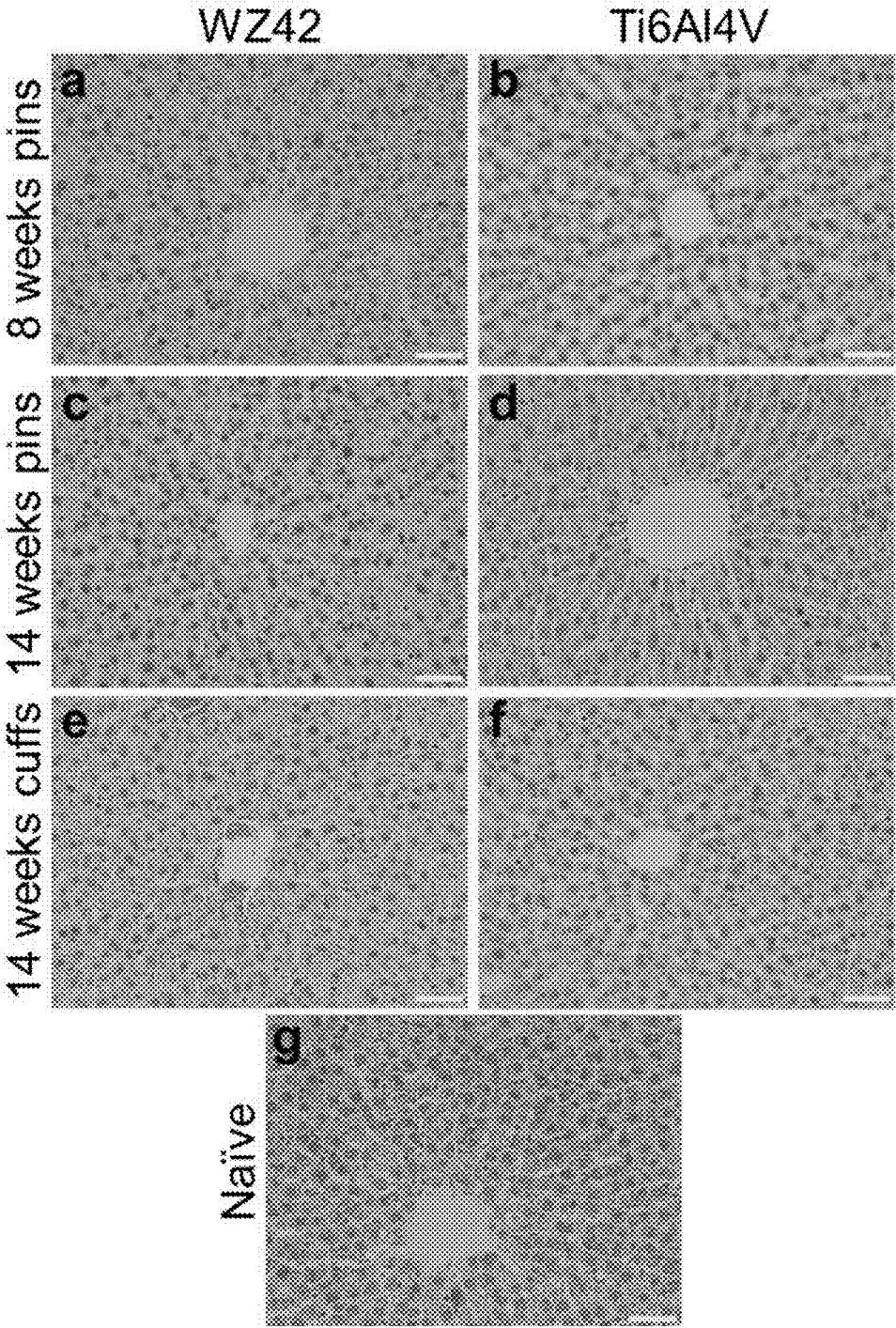


FIGURE 3

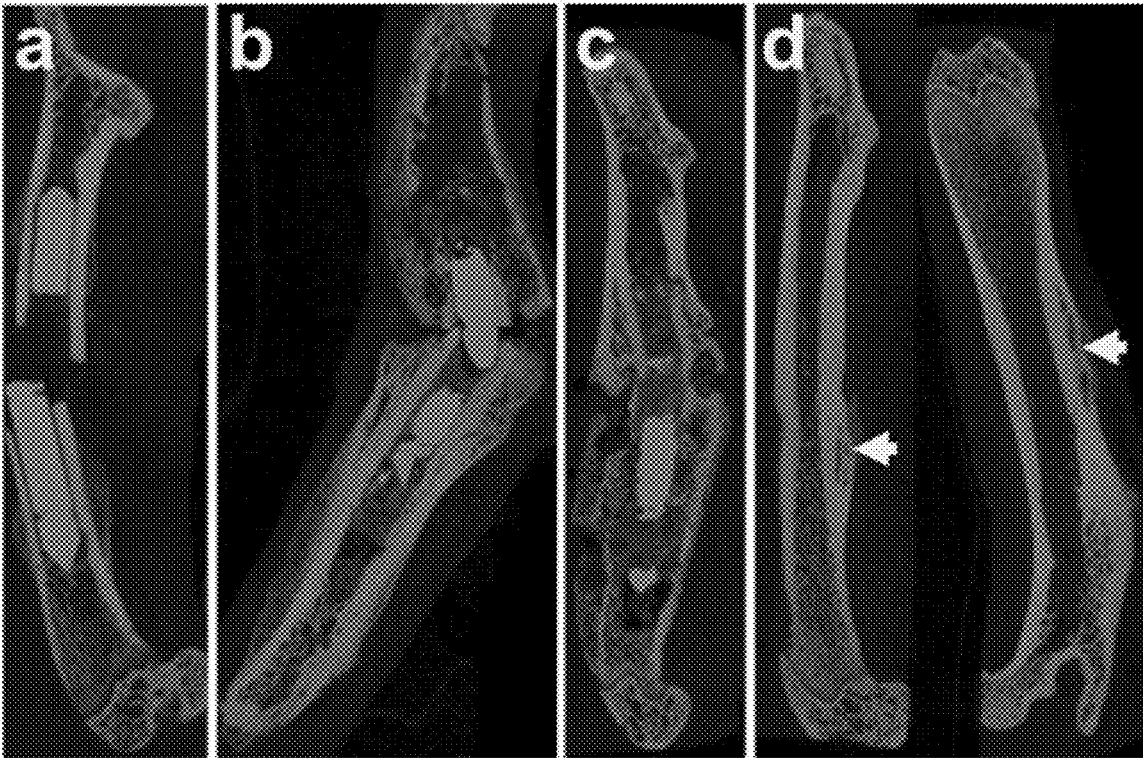


FIGURE 4

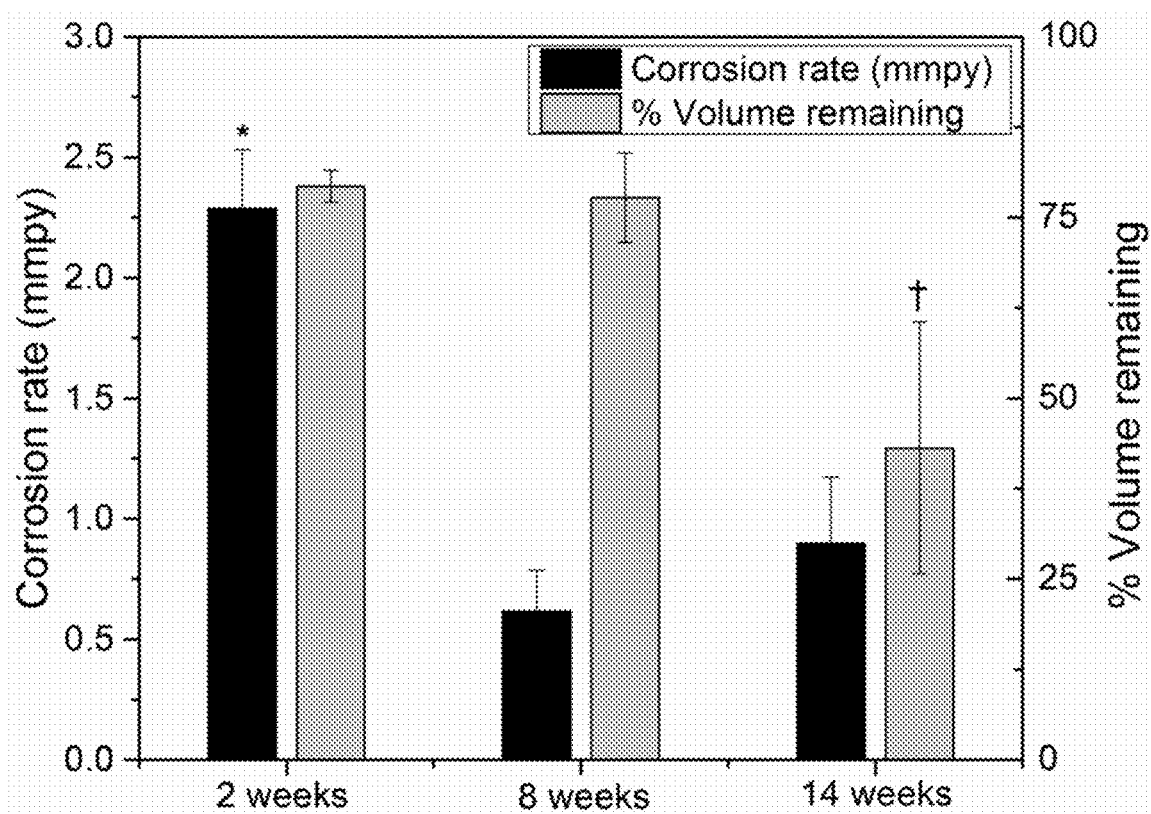


FIGURE 5

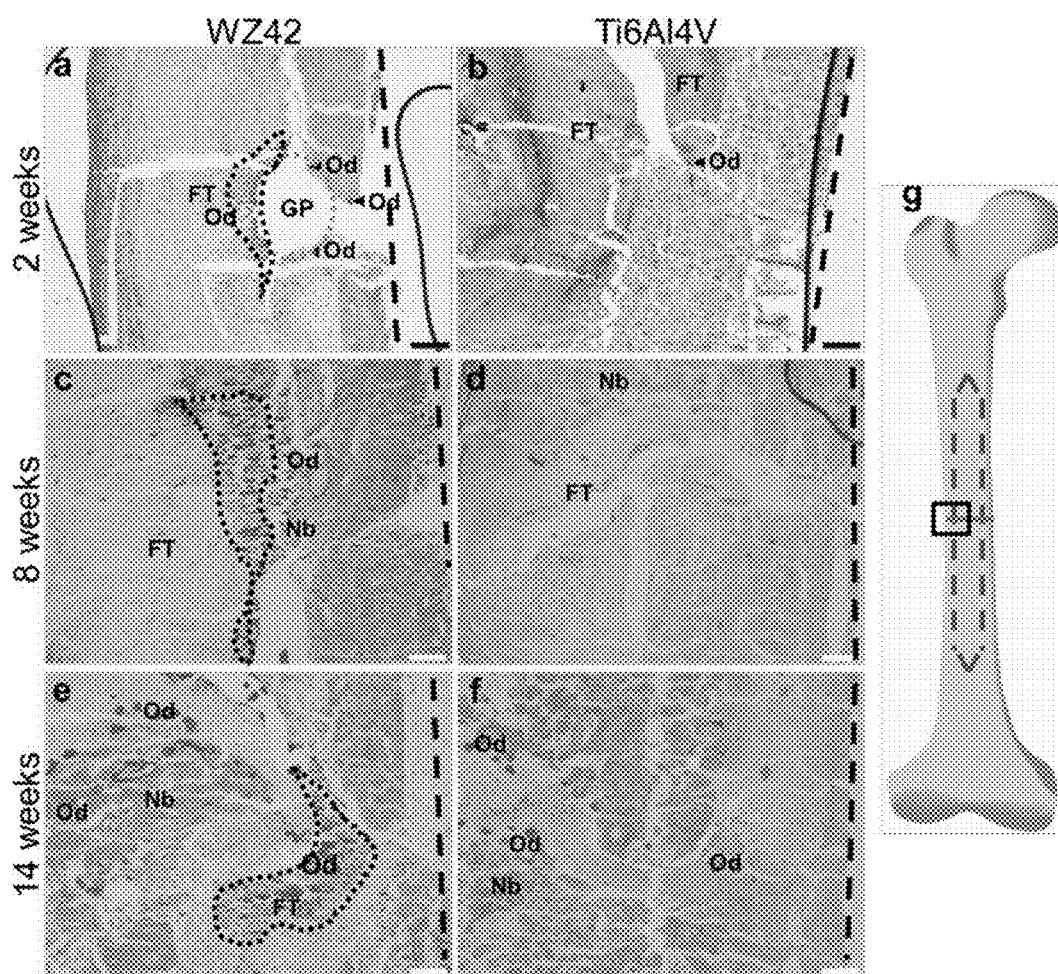


FIGURE 6

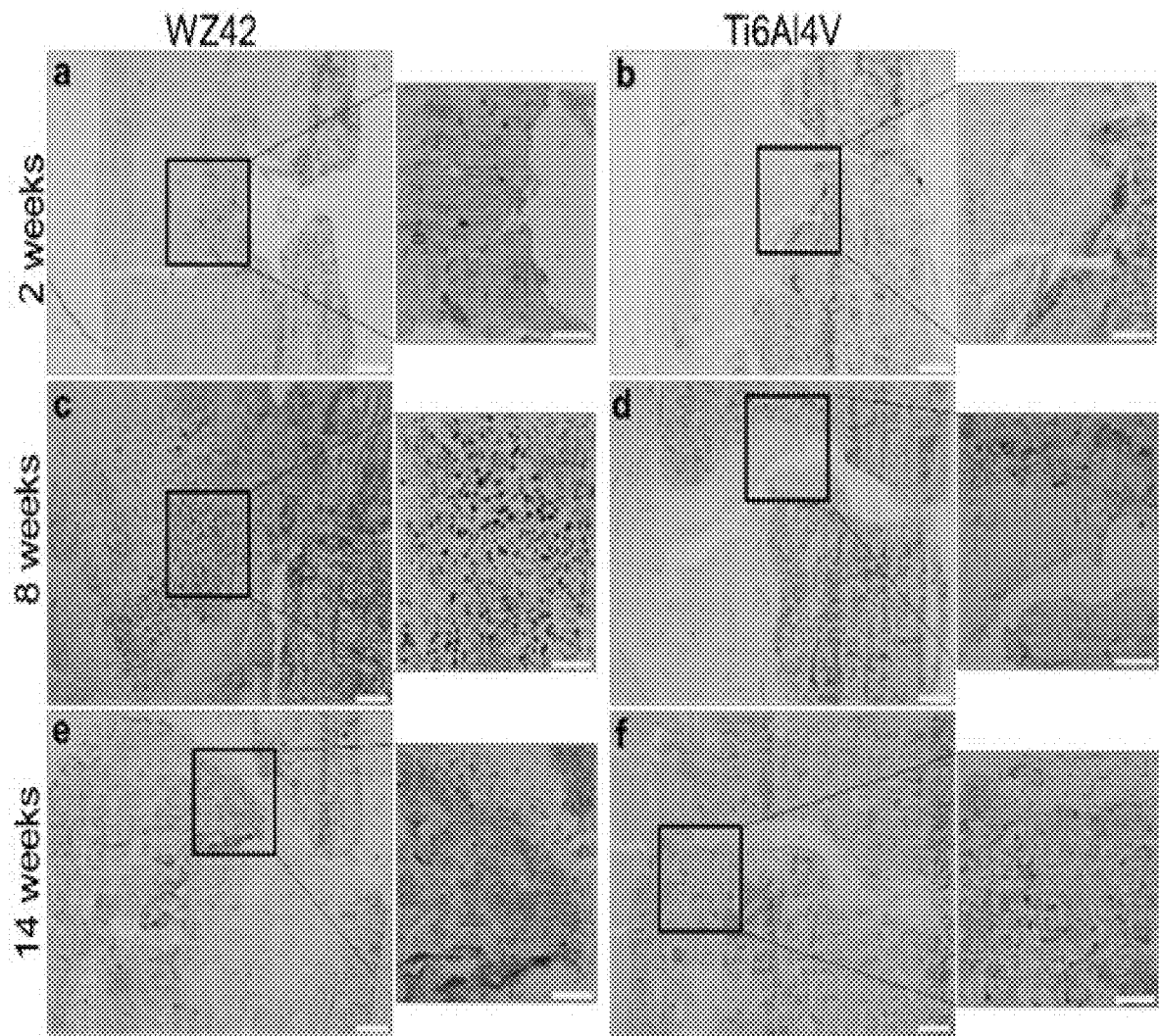


FIGURE 7

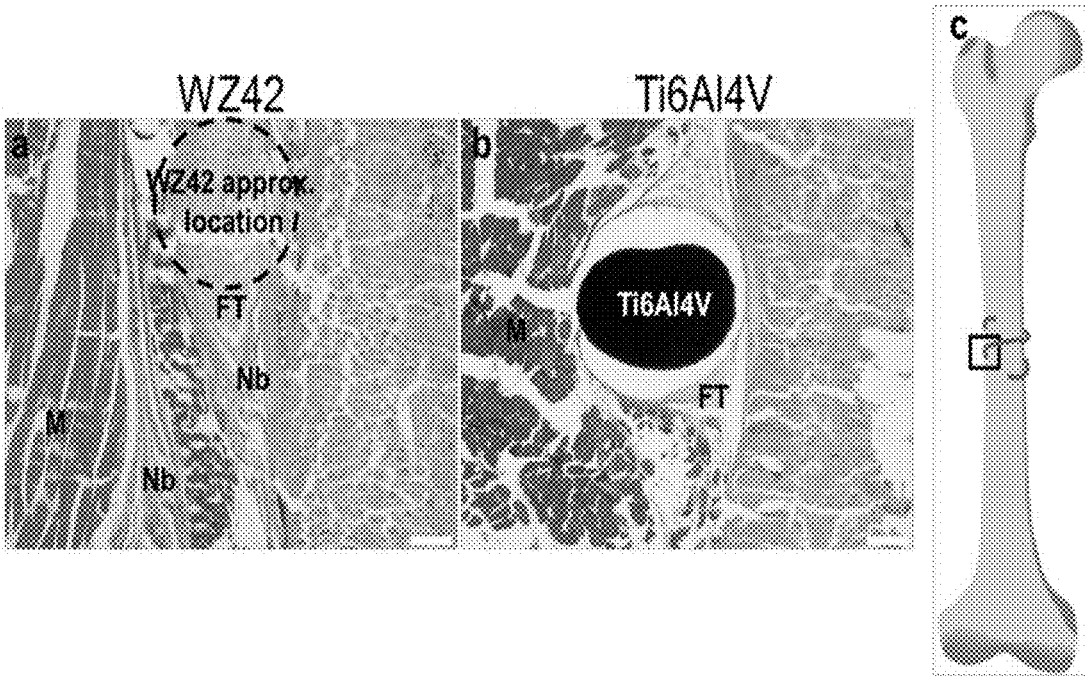


FIGURE 8

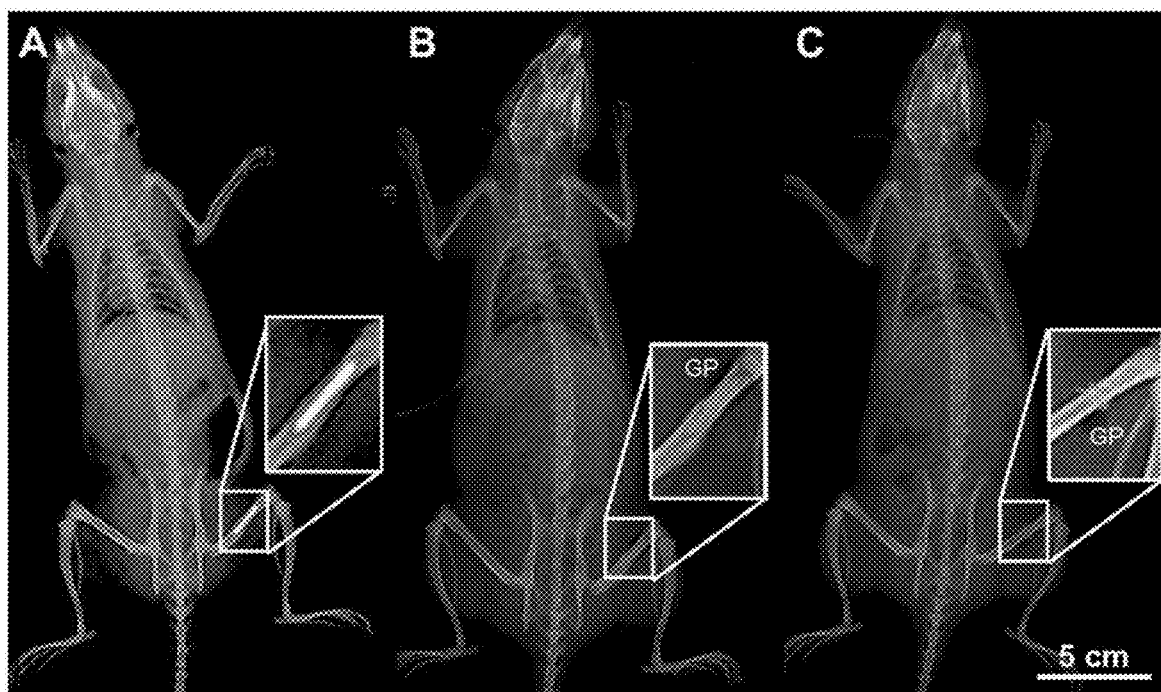


FIGURE 9

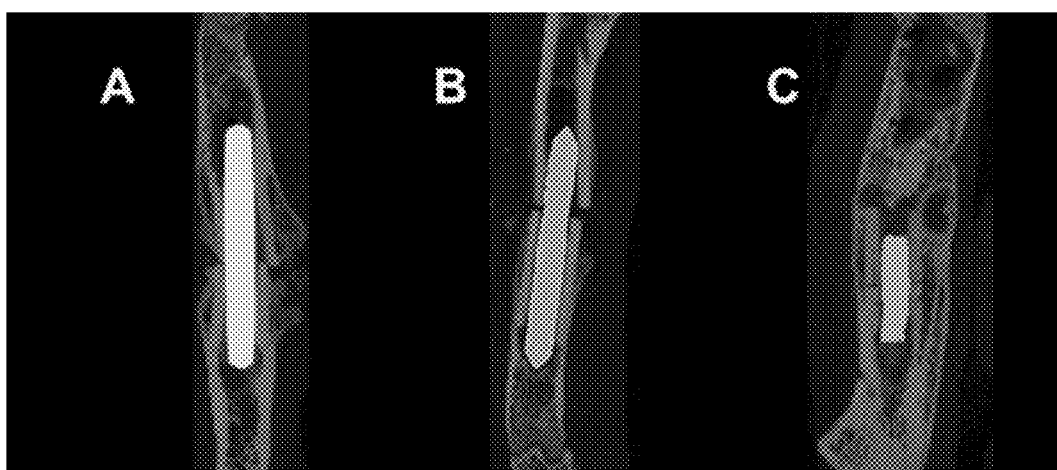


FIGURE 10

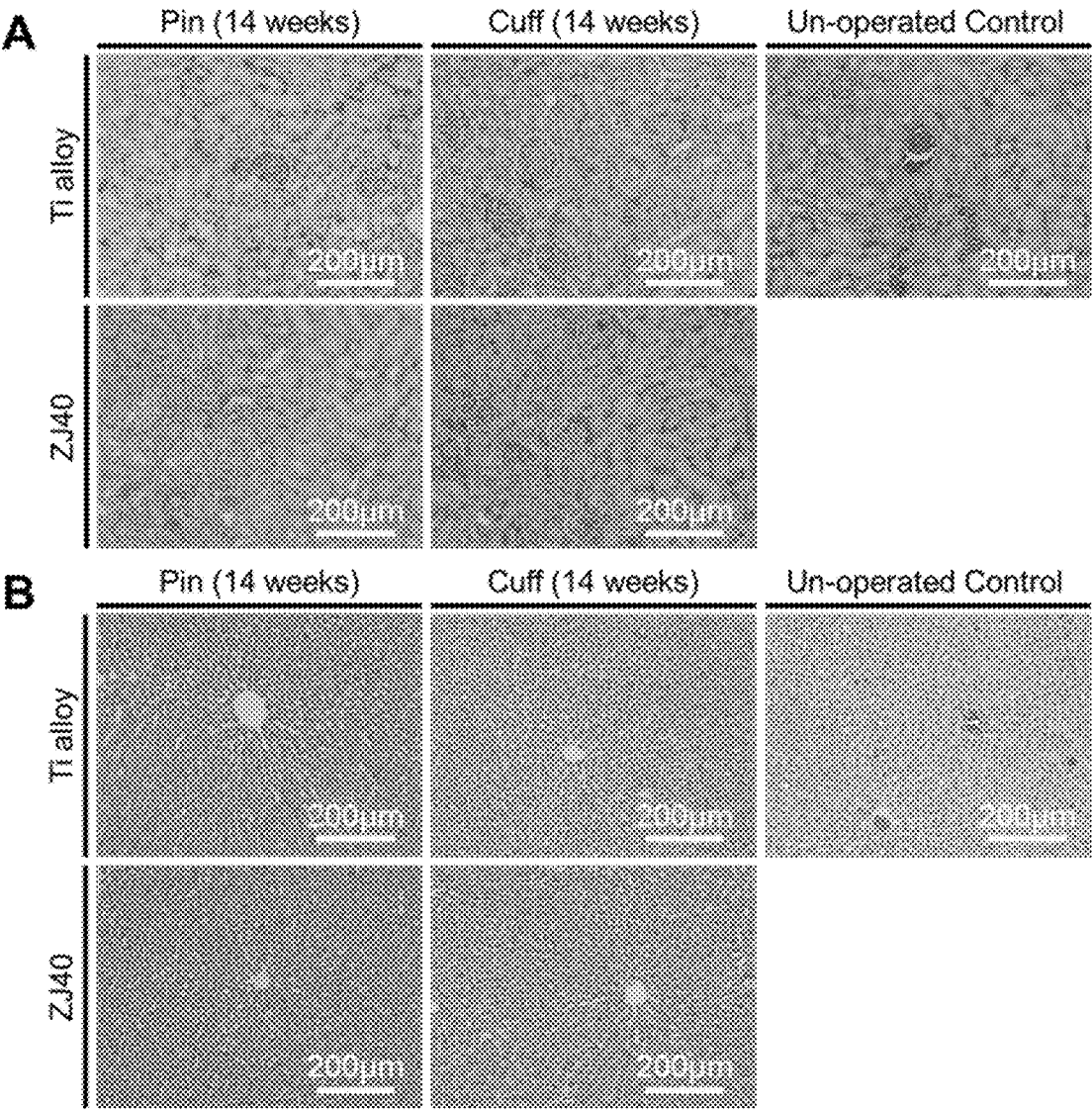


FIGURE 11

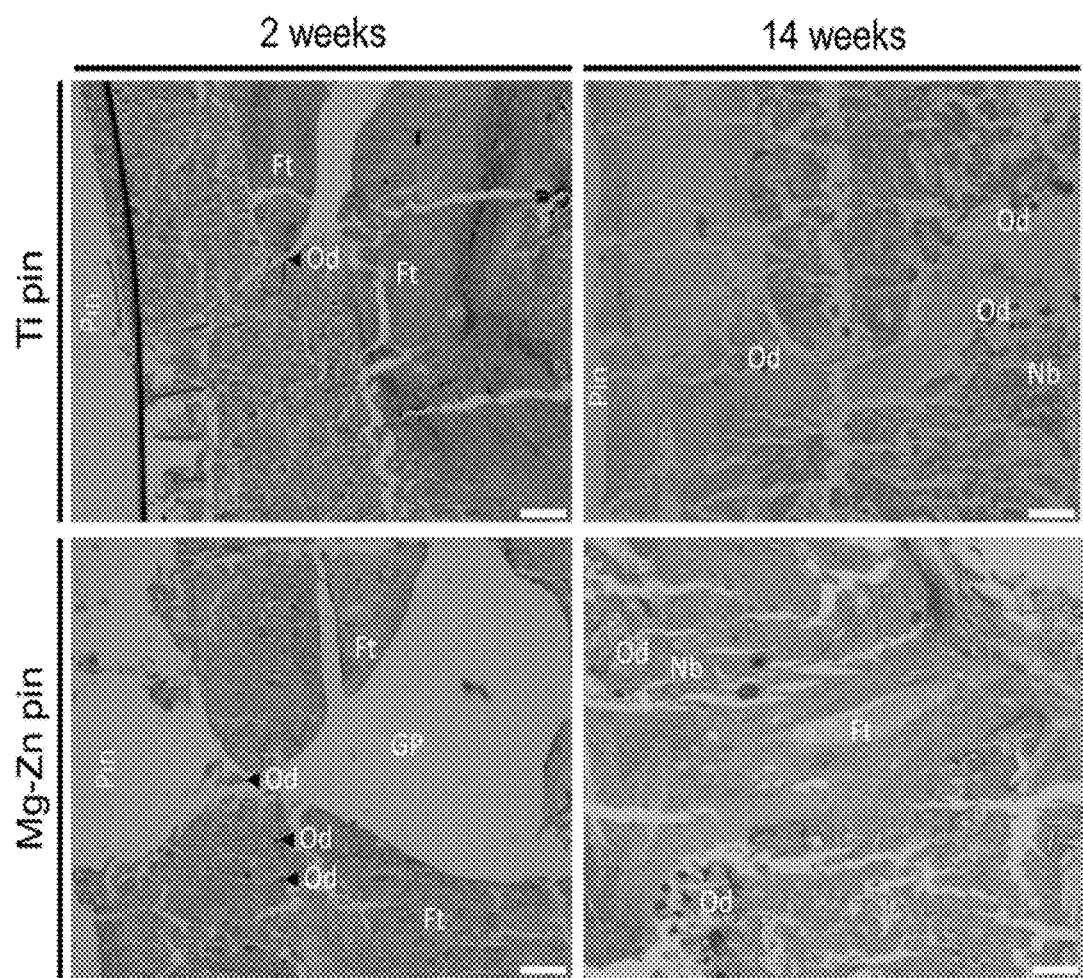


FIGURE 12

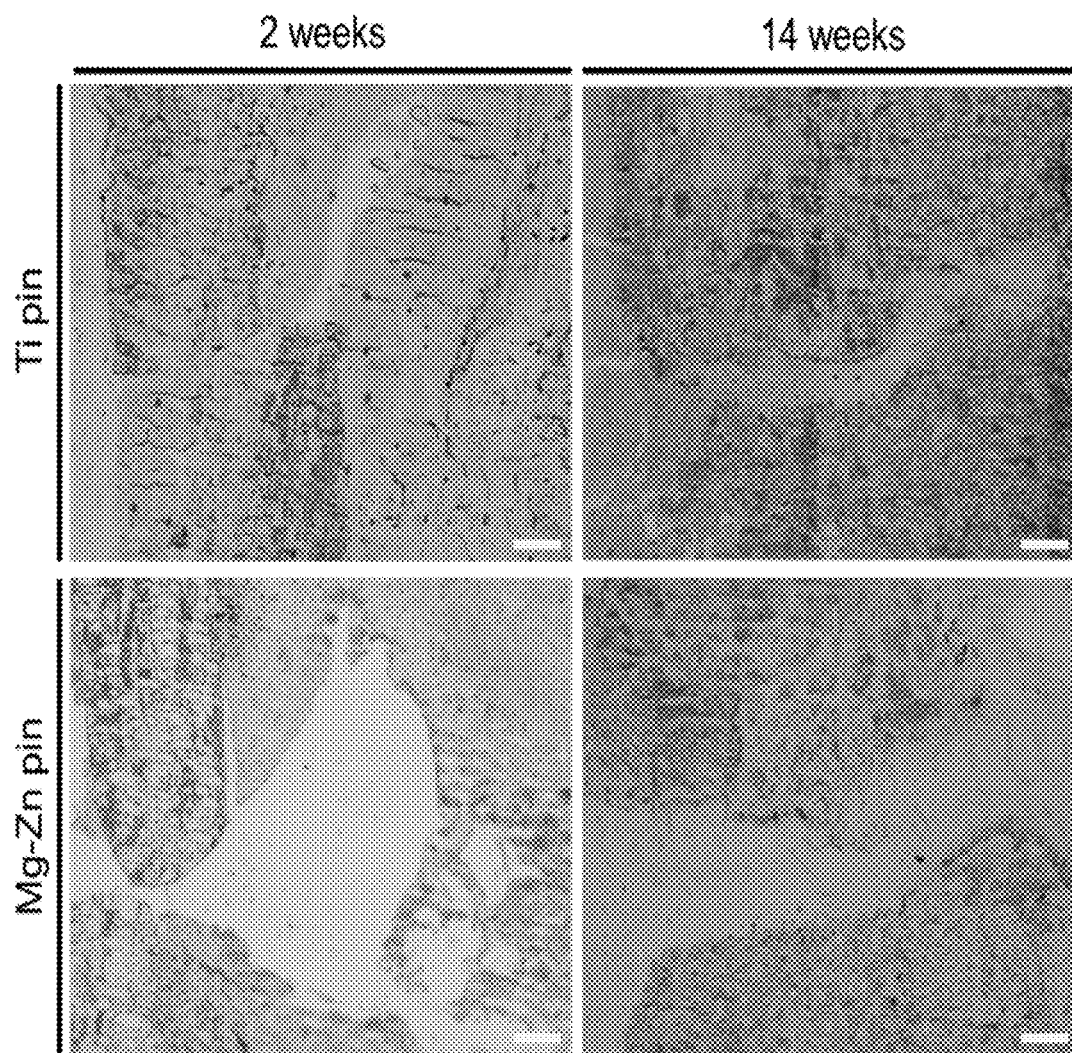


FIGURE 13

DEGRADABLE MAGNESIUM-BASED IMPLANT DEVICES FOR BONE FIXATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. non-provisional patent application Ser. No. 15/750,219, entitled “Degradable Magnesium-Based Implant Devices for Bone Fixation” and filed on Feb. 5, 2018, which claims priority under 35 U.S.C. § 119(e) from U.S. provisional patent application No. 62/208,044, entitled “Degradable Magnesium-Based Implant Devices for Bone Fixation” and filed on Aug. 21, 2015, the contents of which are incorporated herein by reference.

GOVERNMENT SUPPORT AND FUNDING

[0002] This invention was made with government support under grant #0812348 awarded by the National Science Foundation (NSF). The government has certain rights in the invention.

Field of the Invention

[0003] The invention relates to biodegradable magnesium alloy materials, methods for their preparation, and uses as implant devices for bone fixation in load-bearing conditions. The invention is particularly suitable for use in fabricating biodegradable compositions and composites, and medical devices for implantation into a body of a patient, such as for example, orthopedic, craniofacial and cardiovascular implant devices.

BACKGROUND OF THE INVENTION

[0004] Bone fractures generally are common injuries among patients of all ages and may account for more than ten million annual hospital visits in the United States alone. These fractures, small or long bone, are projected to increase due to the occurrence of these injuries in the burgeoning geriatric population with concomitant increases in the prevalence of obesity and osteoporosis. In the pediatric population as well, bone fractures are also extremely common, and may account for about fifteen percent of all injuries.

[0005] Management of bone fractures may require open or closed reduction with or without internal fixation devices, such as, the use of Kirschner wires (K-wires), pins, intramedullary rods or nails, cerclage wires, plates and screws to stabilize bone fragments, and facilitate related healing. K-wires are used by either an open or a percutaneous implantation route to stabilize the fractured fragments by driving a wire between the fractured two pieces of bone to be fixed. The use of K-wires is an established practice for reconstructive surgery, such as, fixation of vascularized free fibular grafts or premaxillary segments in bilateral cleft lip surgery, to stabilize the zygomatic arch fractures or nasal septal fixation in the case of a saddle nose, as well as mandibular reconstruction.

[0006] In bone reconstruction procedures, it is often necessary to hold the bone or fragments of bone together to create a stable environment for tissue and fractured bone healing to occur. This is typically done with metal wires or cables using a technique referred to as the “cerclage”. A cerclage wire or a cable is coiled around a bone or bony fragments to hold them together to allow them to heal. Cerclage has numerous applications in orthopedics particu-

larly, as a primary method for fracture fixation and as a supplement to other forms of hard tissue fixation. Traditionally, these K-wires, pins, nails and cerclage wires are derived from inert and non-degradable cobalt-chromium, stainless steel and titanium alloys. These metals have been traditionally selected for fracture fixation because of their advantageous biomechanical and biocompatibility parameters and characteristics. The high mechanical strength of these metals makes them beneficial for load-bearing applications. However, there have also been disadvantages associated with these metals. For example, a mismatch in mechanical properties compared to cortical bone more often causes stress shielding effects that tend to damage the surrounding hard tissues. Furthermore, these metals have been associated with complications including, but not limited to, pin-tract infections, non-union, mal-union, local soft tissue reaction, nerve injury, broken metal wires or cables, wire migration and morbidity related to secondary procedures or the need for device removal. Degradable polymers such as PLGA/PLL have been used in surgical hardware. However, these devices have been found to be mechanically inferior and not suitable for load bearing (stress, shear, torsional or compressive forces) applications, such as, mandibular and long bone fixation.

[0007] Magnesium alloys have recently emerged as a new class of biodegradable materials for orthopedic applications. Unlike inert titanium or stainless-steel devices, biodegradable Mg hardware is designed to repair bone fractures and resorb over time after bone healing. Compared to conventionally used non-degradable metals, magnesium alloys have biomechanical properties more similar to natural bone. Additionally, magnesium is an essential trace element in the human body and exists in bone naturally as well as has higher mechanical strength compared to biodegradable polymers. Biodegradable magnesium is biocompatible, non-toxic and osteoconductive. Recent reports also suggest osteoinductive characteristics of magnesium. Magnesium alloys are also completely resorbable in the body, which can eliminate the need for complicated and injury prone secondary removal surgeries, and promote enhanced bone reorganization as a result of the gradual increase in load-to-bear associated with the related device degradation.

[0008] Furthermore, the properties of magnesium alloys can be tailored to exhibit desired mechanical and degradation properties matching the defect site for achieving optimal healing. Corrosion is the degradation mechanism common to biodegradable magnesium alloys. During the spontaneous reaction of magnesium with water, magnesium hydroxide and hydrogen gas are produced. Rapid corrosion of magnesium alloys can therefore develop gas pockets near an implantation site and, may lead to patient discomfort and pain due to occurrence of tissue inflammation and infection as a result of the consequent pH change, and the imminent hydrogen gas evolution state. Also, rapid corrosion can cause immature mechanical failure due to loosening of the device following material loss by corrosion during the initial fracture healing process. Thus, to overcome rapid corrosion of magnesium, engineering and design of alloy compositions and their associated microstructure may be tailored with scientific insight by the selection of particular alloy systems and optimization as well as identification of specific processing conditions.

[0009] Although there are non-load bearing fixation devices composed of biodegradable magnesium alloys, there

is a need in the art to design and develop improved biodegradable hardware for implementation in extremities and craniofacial, orthopedic and cardiovascular fixation, such as, resorbable metal-based K-wires, pins and cerclage wires. Therefore, in accordance with the invention, there is provided bioresorbable K-wires, pins, nails and cerclage wired based on novel magnesium alloy compositions, for use under load-bearing conditions, which can circumvent one or more of the above problems associated with indwelling hardware or other fixation devices.

SUMMARY OF THE INVENTION

[0010] In one aspect, the invention provides a biodegradable, magnesium alloy, consisting of from about 0.5 weight percent to about 4.0 weight percent of yttrium; from greater than zero to about 1.0 weight percent of calcium; from about from about 1.0 weight percent to about 6.0 weight percent of zinc; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0 weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

[0011] In another aspect, the invention provides a biodegradable, magnesium alloy consisting of from about 1.0 weight percent to about 6.0 weight percent of zinc; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0 weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

[0012] In still another aspect, the invention provides a biodegradable, magnesium alloy consisting of from about 0.5 weight percent to about 4.0 weight percent of yttrium; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0 weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] A further understanding of the invention can be gained from the following description of the preferred embodiments when read in conjunction with the accompanying figures.

[0014] FIG. 1 is a plurality of images showing one-week post-operative x-ray images of implanted sharpened pins: (a) WZ42 magnesium alloy, (b) fracture misalignment and dead spaces near the WZ42 implants, (c) Ti6Al4V in the right

femur, (d) WZ42 magnesium alloy and (e) Ti6Al4V in the right femur, in accordance with certain embodiments of the invention.

[0015] FIG. 2 is a plurality of photomicrographs of H&E stained kidneys of rats with femurs fixed by pins of WZ42 (a, c) and Ti6Al4V (b, d) after 8 weeks (a, b) and 14 weeks (c, d), stained images of kidneys from rats with implanted wire cuffs WZ42 (e) and Ti6Al4V (f) wrapped around bone for 14 weeks, and (g) of a naïve rat, in accordance with certain embodiments of the invention.

[0016] FIG. 3 is a plurality of photomicrographs of H&E stained livers of rats with femurs fixed by pins of WZ42 (a, c) and Ti6Al4V (b, d) after 8 weeks (a, b) and 14 weeks (c, d), stained images of kidneys from rats with implanted wire cuffs WZ42 (e) and Ti6Al4V (f) wrapped around bone for 14 weeks, and (g) of a naïve rat, in accordance with certain embodiments of the invention.

[0017] FIG. 4 is a plurality of micro-CT scans based on density thresholding with representative cross-sectional slices shown after implantation times of (a) 2 weeks, (b) 8 weeks, and (c) 14 weeks, wherein cuffs were fully degraded after 14 weeks (d) but new bone formation was seen in the region the wires occupied (arrows), in accordance with certain embodiments of the invention.

[0018] FIG. 5 is a bar graph that shows corrosion rate and % volume remaining of WZ42 pins implanted into rat femurs for 2, 8, and 14 weeks, wherein $n \geq 2$ for each group at each time point, * and † represent significant difference ($p < 0.05$) compared to measurements made at other time points, in accordance with certain embodiments of the invention.

[0019] FIG. 6 is a plurality of photomicrographs of Goldner's Trichrome stained sections (40×) of soft and hard tissue at the femoral defect site fixed by pins of WZ42 magnesium alloy (a, c, e) and Ti6Al4V (b, d, f) after 2 weeks (a, b), 8 weeks (c, d), and 14 weeks (e, f) of implantation, (g) representation of region of interest imaged along longitudinal plane at defect site, in accordance with certain embodiments of the invention.

[0020] FIG. 7 is a plurality of photomicrographs of the localization of ALP at 40× and 100× (inset) of tissue at the femoral defect site fixed by pins of WZ42 magnesium alloy (a, c, e) and Ti6Al4V (b, d, f) after 2 weeks (a, b), 8 weeks (c, d), and 14 weeks (e, f) of implantation, in accordance with certain embodiments of the invention.

[0021] FIG. 8 is a plurality of photomicrographs of Goldner's Trichrome stained sections (40×) of soft and hard tissue at the implant-bone interface where wire cuffs of WZ42 magnesium alloy (a) and Ti6Al4V (b) were wrapped around bone for 14 weeks of implantation (c) representation of region of interest imaged along longitudinal plane at defect site, in accordance with certain embodiments of the invention.

[0022] FIG. 9 is a plurality of x-ray radiograph images of (a) Ti pin, (b) Mg-Zn pin and (c) Mg-Zn cuff pin implanted in rats after one week (wherein GP is gas pocket), in accordance with certain embodiments of the invention.

[0023] FIG. 10 is a plurality of micro-CT analysis images of (a) Ti pin at 14 weeks, (b) Mg-Zn pin at 2 weeks, and (c) Mg-Zn pin at 14 weeks, in accordance with certain embodiments of the invention.

[0024] FIG. 11 is a plurality of hematoxylin and eosin staining of harvested (a) liver and (b) kidney, in accordance with certain embodiments of the invention.

[0025] FIG. 12 is a plurality of images of Goldner's Masson Trichrome staining of rat femurs after implantation of Ti and mg-Zn pins for 2 and 14 weeks, in accordance with certain embodiments of the invention.

[0026] FIG. 13 is a plurality of images of alkaline phosphatase staining of rat femurs after implantation of Ti and Mg-Zn pins for 2 and 14 weeks, in accordance with certain embodiments of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0027] The invention relates to novel, biodegradable magnesium alloys, compositions and composites. Furthermore, the invention relates to articles, such as medical devices for implantation into a body of a patient, which are constructed or fabricated from the biodegradable magnesium alloys, compositions, and composites of the invention, for load-bearing conditions. A wide variety of medical implant devices are known in the art and include, but are not limited to, Kirschner wires (K-wires), pins, intramedullary rods or nails, cerclage wires and the like. Moreover, the invention relates to methods of preparing these biodegradable, magnesium alloys, compositions and composites for use in medical applications, such as but not limited to, orthopedic, craniofacial and cardiovascular surgery.

[0028] In addition to biodegradability, the magnesium alloys of the invention include at least one of the following characteristics: biocompatibility, corrosion resistance, cell attachment, viability and mechanical strength. The magnesium alloys can include alloying elements that are pre-selected and presented in particular amounts, such that one or more desired characteristics, such as, but not limited to biodegradation and mechanical strength, may be achieved.

[0029] In addition, to component selection in specific percentages, desired characteristics, such as but not limited to, biodegradation and strength of the magnesium alloys can be controlled or tailored by appropriate post-processing and microstructural modifications. For example, following melting and casting of the magnesium alloys, post-processing steps of solution treatment and hot extrusion can be implemented to impart desired grain refinement to further improve and augment the mechanical properties, and promote corrosion resistance of the magnesium alloys.

[0030] The magnesium alloys can contain unique combinations of elements, which include precipitates arranged in a long period stacking order (LPSO), resulting in high strength.

[0031] In certain embodiments, the invention includes controlling corrosion rate and improving mechanical properties of magnesium alloys through the introduction of additional, e.g., alloying, elements and optimal processing conditions. It has been found that magnesium corrosion and mechanical properties are affected by specific alloying elements.

[0032] In certain embodiments of the invention, the biodegradable, magnesium alloys include alloying elements selected from yttrium, calcium, zinc, zirconium and strontium. As stated above herein, the specific alloying elements selected and the amounts of each can vary. In general, the amounts of each of the alloying elements are selected such that the resulting compositions are within acceptable non-toxic limits, sufficiently biocompatible for implantation into a body of a patient, and degradable over a period of time so that the implantation device does not remain in the body of

the patient for prolonged periods of time, e.g., not beyond the period of time when there is a medical need for the implantation device. An implantation device fabricated in accordance with the invention will degrade and preferably completely dissolve within an acceptable time frame. Acceptable non-toxic limits and degradation can vary and can depend on the particular physical and physiological characteristics of the patient, the particular in vivo site of the implantation device, and the particular medical use of the implantation device.

[0033] In certain embodiments, the magnesium alloys include yttrium, zinc, calcium and zirconium; or yttrium, zinc, calcium, zirconium and strontium; or yttrium, zinc and zirconium; or yttrium, zinc, zirconium and strontium; or yttrium, zirconium and strontium, with a remainder of magnesium and impurities due to the production. In these embodiments, yttrium constitutes from about 0.5 weight percent to about 4.0 weight percent; calcium constitutes from greater than zero to about 1.0 weight percent; zirconium constitutes from greater than zero to about 1.0 weight percent; zinc constitutes from about 1.0 weight percent to about 6.0 weight percent; strontium constitutes from greater than zero to about 6.0 weight percent or from about 0.10 weight percent to about 6.0 weight percent, with the remainder being magnesium and impurities due to production, based on the total weight of the alloy.

[0034] In one embodiment, the biodegradable, magnesium alloy consists of from about 0.5 weight percent to about 4.0 weight percent of yttrium; from greater than zero to about 1.0 weight percent of calcium; from about 1.0 weight percent to about 6.0 weight percent of zinc; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0 weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

[0035] In another embodiment, the biodegradable, magnesium alloy consists of from about 1.0 weight percent to about 6.0 weight percent of zinc; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0 weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

[0036] In still another embodiment, the biodegradable, magnesium alloy consists of from about 0.5 weight percent to about 4.0 weight percent of yttrium; from greater than zero to about 1.0 weight percent of zirconium; from greater than zero to about 6.0 weight percent of strontium; optionally from about 1.0 weight percent to about 9.0 weight percent aluminum; optionally from about 0.1 weight percent to about 1.0 weight percent of manganese; optionally from about 0.25 weight percent to about 1.0 weight percent of silver; optionally from about 0.1 weight percent to about 1.0

weight percent of cerium; and a balance of magnesium and impurities due to production, based on total weight of the alloy.

[0037] Without intending to be bound by any particular theory, it is believed that the presence of yttrium can contribute to the improved mechanical strength and corrosion resistance; calcium can prevent oxidation during the casting of the alloy; zirconium can act as a grain refiner and improve mechanical properties of the compositions; and strontium can stimulate bone formation. The amounts of magnesium and the alloying elements may be specified and adjusted such as to control at least one of corrosion resistance, biodegradation, biocompatibility, toxicity, cell attachment, mechanical strength and flexibility characteristics.

[0038] Furthermore, in certain embodiments, one or more other alloying elements may be added to the magnesium alloys to impart additional characteristics and properties. These other alloying elements can be selected from those known in the art, and can include, but are not limited to, cerium, aluminum, manganese, and silver, in amounts that can vary. One or more of cerium, aluminum, manganese and silver may be present in the magnesium alloy, wherein each is present in an amount of from greater than zero to about 1.0 percent by weight based on total weight of the alloy.

[0039] In certain embodiments, aluminum constitutes from about 1.0 weight percent to about 9.0 weight percent based on the total weight of the alloy. In other embodiments, aluminum constitutes from about 2.0 weight percent to about 9.0 weight percent based on the total weight of the alloy.

[0040] In certain embodiments, manganese constitutes from about 0.1 weight percent to about 1.0 weight percent based on the total weight of the alloy. In other embodiments, manganese constitutes from about 0.2 weight percent to about 1.0 weight percent based on the total weight of the alloy.

[0041] In certain embodiments, silver constitutes from about 0.10 weight percent to about 1.0 weight percent based on the total weight of the alloy. In other embodiments, silver constitutes from about 0.25 weight percent to about 1.0 weight percent based on the total weight of the alloy.

[0042] In certain embodiments, cerium constitutes from about 0.1 weight percent to about 1.0 weight percent based on the total weight of the alloy. In other embodiments, cerium constitutes from about 0.5 weight percent to about 1.0 weight percent based on the total weight of the alloy.

[0043] Non-limiting examples of medical devices in which the magnesium alloys, compositions and composites of the invention can be used include, but are not limited to wires, such as, Kirchner wires (K-wires), pins, intramedullary rods or nails, cerclage wires and related devices, including, plates, meshes, staples, screws, tacks, suture anchors, tubular mesh, coils, x-ray markers, catheters, endoprostheses, pipes, shields, bolts, clips or plugs, dental implants or devices, graft devices, bone-fracture healing devices, bone replacement devices, joint replacement devices, tissue regeneration devices, cardiovascular stents, intercranial aneurism device, tracheal stents, nerve guides and surgical implants.

[0044] The biodegradable, magnesium alloys of the invention can be prepared using various methods and processes. In general, conventional melting and casting methods, and processes are employed. It is known in the art of metallurgy that casting is a production technique in which a metal or a

mixture of metals, e.g., metal alloy, is heated until molten and then poured into a mold, allowed to cool, and thereby solidified. In certain embodiments, a melted or molten mixture of metals or metal alloy is poured into a mild steel/copper mold at a temperature from ambient, e.g., room temperature, to 500° C.

[0045] Casting of the magnesium-based alloys of the invention can be carried out using any conventional casting procedures known in the art, such as, but not limited to, sand casting, gravity casting, permanent mold casting, direct chill casting, centrifugal casting, low/high pressure die casting, squeeze casting, continuous casting, vacuum casting, plaster casting, lost foam casting, investment casting, and lost wax casting. It is believed that the particular process used for casting may affect the properties and characteristics of the cast alloy. Furthermore, it is believed that the temperature at which the melting procedure is performed may also affect the alloy. Thus, the temperature may be carefully selected so as to maintain or achieve desired properties of the magnesium alloy.

[0046] The resulting cast can be subjected to various forming and finishing processes known in the art. Non-limiting examples of such processes include, but are not limited to, solution treating, quenching, extruding, e.g., hot extruding, homogenizing, forging, rolling, equal channel angular extrusion, stamping, deep-drawing, wire drawing, polishing (by mechanical and/or chemical means), surface treating (to form a superficial layer on the surface), machining, e.g., lathe machining, and combinations thereof.

[0047] In certain embodiments, the invention includes melting (e.g., heating at an elevated temperature) and casting the magnesium and alloying elements, followed by solution treatment and hot extrusion. The hot extrusion can be carried out at a temperature of about 450° C. with an extrusion ratio of 30:1. The extruded alloy can be machined, such as, by lathe machining, to form an implant device, e.g., pins or wires. The implant device is typically washed, dried and sterilized prior to implantation into the patient.

[0048] Furthermore, prior to solidification, the molten mixture or alloy may be tested to determine the amount of the various components therein and therefore, to provide an opportunity to adjust the amounts as desired prior to solidification.

[0049] One or both of the melting and casting steps may be performed under a protective atmosphere to preclude, minimize or reduce oxidation/decomposition of the elements in the material. In particular, it is desirable to preclude, minimize or reduce the oxidation/decomposition of magnesium. The protective atmosphere can include, but is not limited to, argon, sulfur hexafluoride, carbon dioxide, dry air and mixtures thereof.

[0050] In certain embodiments, subsequent to the casting process, the magnesium-containing cast may be subjected to homogenization. Without intending to be bound by any particular theory, it is believed that homogenization treatment can cause the spreading of, or creation of more even or uniform distribution of, impurities, secondary phase(s), and inter-metallic phases, if present therein. The resulting alloy may be in the form of a single phase, solid solution.

[0051] Detailed exemplary procedures for melting, casting, solution treating, and extruding are depicted in the following examples.

[0052] Additional objects, advantages and novel features of the invention may become apparent to one of ordinary

skill in the art based on the following examples, which are provided for illustrative purposes and are not intended to be limiting.

EXAMPLES

Example 1

[0053] This experiment evaluated the safety and efficacy of a novel Mg-Y-Zn-Zr-Ca alloy compared to non-degradable Ti6Al4V, over a 14-week follow-up, implanted as pins to fix a full osteotomy in rat femurs and as wires wrapped around the outside of the femurs as a cerclage. To assess systemic toxicity, blood cell count and serum biochemical tests were performed. Livers and kidneys were harvested to observe histomorphological alterations and accumulation of alloying elements. Hard and soft tissue adjacent to the fracture site were examined using Goldner's Trichrome and alkaline phosphatase staining. Degradation behavior of the Mg alloys was determined using μ CT scans to assess alloy degradation and bone morphology. Blood testing exhibited no significant changes arising from the Mg alloy compared to the control groups. No recognizable differences in the morphology of liver and kidney tissue, and no accumulation of Mg, Zn, and Ca in these organs were observed. Corrosion occurred gradually, with degradation slowing after 2 weeks, with points of high stress observed near the fracture site ultimately resulting in Mg alloy pin fracture. Nevertheless, normal bone healing was observed in femurs fixed with the Mg alloy that was confirmed by the presence of osteoids, osteoblast activity, and new bone formation. These results demonstrate the feasibility of the Mg-Y-Zn-Zr-Ca alloy for orthopedic and craniofacial fracture fixation applications.

[0054] A fully load-bearing model was employed, wherein a full fracture of the rat femur was fixed using only the implant pins of the Mg alloy, Mg-Y-Zn-Zr-Ca (WZ42), and the performance was compared to pins machined from the common medical titanium alloy, Ti6Al4V. The model bears the characteristics of exposing the system to considerable stress in the absence of any external immobilization thereby conducive to biodegradation via stress induced corrosion, since the animal is allowed to ambulate immediately following surgery combined with dual exposure to the vasculature creating therefore ideal conditions for stress corrosion. The model is therefore ideal to evaluate intrinsic upper bound toxicity of the alloy due to acceleration of the corrosion process via stress induction thus creating a condition inducing maximum dissolution of the alloy and the alloying elements. Favorable outcome of the alloy with no ensuing tissular toxicity under these conditions is a true validation of the biocompatibility and biodegradable attributes of the alloy composition. Moreover, this model selected has conceptual similarities mimicking orthopedic fixation devices such as Kirschner wires (K-wires) and Steinmann pins—thin rods that are drilled or tapped through bone fracture fragments to maintain the anatomical congruity and biomechanical stability required for optimal bone healing. Currently used stainless steel and titanium K-wires are removed after the bone has healed, necessitating a secondary painful removal procedure the patient must endure. To allow for easy removal of the K-wire during this secondary procedure, the ends of the rods are usually left outside the skin, forming a “pin-tract” that may inevitably act as a conduit for causing infection. Other complications arising from these fixation devices include nerve injury,

pain, osteomyelitis, and migration. These shortcomings of K-wires and other common orthopedic devices derived customarily from existing inert metals could thereby be avoided through the use of degradable Mg alloys detailed in this invention.

[0055] In addition to this challenging load-bearing model with intramedullary fracture fixation pins, WZ42 wires were wrapped around the mid-diaphyseal region of unfractured femurs forming a cerclage cuff to compare degradation and tissue response to the Mg alloy implanted in different regions—intramedullary versus over the cortical bone. With these two implants, in vivo corrosion, bone healing, and host response was assessed to provide an overall evaluation of the degradable WZ42 magnesium alloy when utilized in orthopedic device applications.

Materials and Methods

Preparation of Mg-Y-Zn-Zr-Ca Implants

[0056] The procedure for melting and casting the WZ42 alloy (nominal composition of Mg-4.0% Y-2.0% Zn-1.0% Zr-0.6% Ca in wt. %) was conducted. Alloying elements in pure form and contained in Mg master alloys were melted in an electrical resistance furnace (Wenescor Inc., Chicago, IL) under the protection of Ar+1.5% SF₆ cover gas and cast into a cylindrical mild steel mold preheated to 500° C. after stirring and holding for 30 minutes to achieve dispersion of Zr. After casting, a solution treatment of 400° C. was applied for 20 hours and the ingot was quenched to room temperature in water to increase the ductility of the alloys and homogenize the secondary phases. The ingot was extruded at a temperature of 450° C. with an extrusion ratio of 30. The extruded WZ42 and control material Ti6Al4V (Goodfellow Corporation, Coraopolis, PA) were lathe machined into pins with dimensions of 15 mm length×1.66 mm diameter and, wires of 20 mm length and 0.68 mm diameter. The implants were sonicated in washes of acetone and isopropanol and dried before undergoing sterilization by gamma radiation (2×10⁶ cGy, 23.5 Gy/min, cesium 137 source, Mark I 68, JL Shepherd and Associates, San Fernando, CA).

Surgical Model and Study Protocol

[0057] All the animal experiments were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC). Before surgery, female Sprague-Dawley rats weighing 250-300 g were anesthetized by inhalation of isoflurane at a concentration of 2-5% for initiation of sedation, and 0.25-4% for maintenance. Only the right hind limb of each rat was operated. First, the right hind limbs were shaved and disinfected, and an approximately 2 cm incision was made over the dorsolateral right femur. The skin and mid-diaphyseal region of the right femurs were exposed through a lateral approach. A complete femoral osteotomy was created using a circular saw. The WZ42 or Ti6Al4V fixation pins were inserted first into the intramedullary space of the distal portion of the fractured femur, then inserted into the intramedullary space of the proximal femur, with the fracture approximated. In the case of the wire cuffs, the right femur was not cut, and the wires were wrapped around the midsection of the diaphysis over the periosteum and pressed against the bone to avoid any translation along the shaft of the femur or migration. After the samples were implanted, the fascia and muscles were closed with 3.0

VICRYL (J315), and the skin closed using non-absorbable monofilament 3.0 polyamide sutures.

[0058] Post-operative pain and distress was observed daily for expressions of stress and behavioral abnormalities, changes in movement, food, and water intake. Furthermore, the right hind limbs were clinically observed on a daily basis for signs of infection, wound dehiscence, presence of gas pockets or abnormal posture/thigh anatomy.

[0059] Groups of five animals for both WZ42 and Ti6Al4V pins were used for each time point of 2, 8, and 14 weeks postoperative for blood values, tissue samples (liver, kidney, femurs with surrounding soft tissues), and micro-CT analysis, and groups of 6 animals were implanted with wire cuffs with a single time point of 14 weeks for toxicological assessment, as displayed in Table 1.

TABLE 1

Summary of number of rats in each group at time points used in study.					
	Time point:				
	Pre-operative	2 weeks	8 weeks	14 weeks	14 weeks
		Intramedullary Pin			Cuff
Ti6Al4V		5	5	5	6
WZ42		5	5	5	6
Naïve	3				

[0060] Immediately following sacrifice, the liver, kidney, and experimental group femurs were collected and stored for further analysis as described in the following sections. Three rats receiving no surgery were also sacrificed to serve as the naïve control group.

X-Ray Imaging

[0061] Conventional X-ray imaging was performed on rats one week post-operatively to examine the position of the implants and stability of the fracture. For that purpose, the animals were anesthetized with isoflurane.

Blood-cell Count and Serum Biochemical Measurements

[0062] Blood samples were collected from animals before operation (baseline) under anesthesia by tail snip and terminally (2, 8, and 14 weeks post-implantation) by cardiac puncture. Whole blood cell counts were performed by Marshfield Labs (Cleveland, OH) using a Sysmex XT2000i Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan) with provided reference ranges. Serum samples were obtained by centrifuging collected blood at 2,000 rpm for 10 minutes at 4° C. Serum biochemical tests were conducted by Marshfield Labs using an Olympus AU chemistry analyzer (Olympus Corporation, Tokyo, Japan) with reported reference ranges established by Marshfield Labs.

Micro-computed Tomography Imaging

[0063] Plastic embedded rat femurs were used for high resolution micro-computed tomography (μ CT) scanning. WZ42 alloy samples were scanned with continuous rotation μ CT at 10.5 μ m voxel size before implantation and, after retrieval along with surrounding tissue post-operatively at 2, 8, and 14 weeks. The reconstructed data sets were used to generate a 3D volume from which the remaining metal rod

was distinguished from the surrounding degradation products and bone by using a histogram of grey values based on densities. A density threshold for the metal pins was used to isolate the volume of remaining magnesium alloy from the surrounding material and compared to the volume of the pins before implantation to estimate in vivo corrosion rate using the following equation adapted from the standard ASTM G31:

$$C=(K \times V)/(A \times T)$$

wherein C is the corrosion rate (mm year^{-1} or mmpy), the constant K is 8.76×10^4 , V is the volume loss (cm^3), A is the initial sample area exposed (cm^2), and T is the time of exposure (h).

Histological Preparation and Analysis

[0064] Specimens of liver and kidney were fixed in 10% neutral buffered formalin, dehydrated, then infiltrated and embedded in paraffin. They were stained with hematoxylin and eosin (H&E) and evaluated for cell infiltration, tissue morphology and pathological changes due to degradation and clearance of the WZ42 alloy in these critical visceral organs.

[0065] Femurs were fixed in 70% ethanol, dehydrated, and infiltrated and embedded in Osteo-Bed Plus methyl methacrylate-based embedding kit (Polysciences, Inc., Warrington, PA). The plastic blocks were sectioned with a rotary microtome (Leica RM 2255, Leica Biosystems, Buffalo Grove, IL) and stained using Goldner's Trichrome and alkaline phosphatase stains to observe bone morphology and, osteoblast activity at the site of fracture and surrounding the implants.

Tissue Digestion and Elemental Analysis

[0066] Liver and kidney tissues were digested to allow for measurement of elemental concentration using inductively coupled plasma with optical emission spectroscopy (ICP-OES). First, tissues were dried at 70° C. for 24 hours, then homogenized and weighed. The samples were then digested by immersion in 20 ml nitric acid/g tissue for 6 hours at 70° C., followed by the addition of 4 ml hydrogen peroxide/g tissue for 1 hour and 4 ml sulfuric acid/g tissue for 1 hour. Samples were then diluted 50x in ultra-pure deionized water (purified using Milli-Q Academic, Millipore, Billerica, MA), filtered in 0.45 μ m syringe filters, and analyzed for Mg and alloying element concentration by ICP-OES (ICP-OES, iCAP duo 6500 Thermo Fisher, Waltham, MA).

Statistical Analysis

[0067] Statistical analysis was conducted using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). Differences between the groups were analyzed using one-way ANOVA with post-hoc testing using Gabriel's pairwise test. $P < 0.05$ was accepted as a statistically significant difference between means.

Results

Fixation of Femoral Fracture Using Mg-Y-Zn-Zr-Ca Alloy Pin

[0068] The intramedullary pins were successfully inserted into the fractured femurs during surgery with the fractures being approximated as seen in the 1-week postoperative

X-ray images of FIG. 1 *a-c*, despite the slight mismatches between the pin diameter and the diameter of the intramedullary cavity observed in 68% (13 of 19) of cases (FIG. 1*b*, arrow) owing to manual surgical placement resulting in a small gap or misalignment in the two sides of the fractured femur. All the wire cuffs maintained their position wrapped around the femur as shown in FIG. 1*d* and *e*.

[0069] Small pockets of dead space (FIG. 1*b*, arrow) were observed in the 1-week X-rays of 73% (19 of 26) of the rats with implanted WZ42 alloy pins or cuffs, likely caused by hydrogen gas evolved from the degrading Mg implants.

Despite their presence in the X-ray images, no bulges in the skin in the hind limb of the rats were observed during the frequent visual inspections of the rats. The rats had regained mobility by postoperative day 7.

Systemic Toxicity to Mg-Y-Zn-Zr-Ca Implants

[0070] Total blood cell counts are listed in Table 2, which generally did not reveal any disturbance in the blood count values, with parameters remaining within references ranges or near pre-operation levels.

TABLE 2

Average blood cell counts of naïve animals and animals implanted with WZ42 and Ti6Al4V alloy pins and cuffs at 2, 8, and 14 weeks after implantation.					
Name	Implantation time	Red Blood Cell Count	Hemoglobin	Platelet Count	White Blood Cell Count
Units		10 ⁶ /uL	g/dL	10 ³ /uL	10 ³ /uL
Ref. ranges		(7.00-9.00)	(13.7-16.8)	(680-1280)	(1.1-7.5)
Naïve		7.4 ± 0.3	14.1 ± 0.9	618.3 ± 200.6	6.8 ± 2.3
WZ42 pin	2 weeks	8.0	14.3	839.0	8.6
Ti6Al4V pin	2 weeks	7.8	14.9	656.0	9.0
WZ42 pin	8 weeks	7.4 ± 0.5	13.8 ± 1.2	N/A	6.8
Ti6Al4V pin	8 weeks	7.4 ± 0.3	14.2 ± 0.4	637.8 ± 168.6	5.9 ± 1.6
WZ42 pin	14 weeks	7.7 ± 0.4	14.2 ± 0.5	595.8 ± 179.8	5.9 ± 2.1
Ti6Al4V pin	14 weeks	7.5 ± 0.4	14.0 ± 0.7	563.0 ± 164.5	5.9 ± 2.7
WZ42 cuff	14 weeks	7.1 ± 0.2	13.9 ± 0.4	461.0 ± 56.6	2.2 ± 1.0
Ti6Al4V cuff	14 weeks	7.6 ± 0.4	13.8 ± 0.7	637.0 ± 96.5	5.8 ± 1.4

[0071] Small differences from the reference ranges or naïve levels were observed for low platelet counts in the WZ42 cuff group, and WZ42 and Ti6Al4V pins at 14 weeks. Elevated postoperative white blood cell counts were seen at 2 weeks for both WZ42 and Ti6Al4V pins.

TABLE 3

Average values of serum metabolic parameters of naïve animals and animals implanted with WZ42 and Ti6Al4V alloy pins and cuffs at 2, 8, 14 weeks after implantation.						
Name	Implantation time	Glucose	ALT(GPT)	ALP	Total Bilirubin	Total Protein
Units		mg/dL	U/L	U/L	mg/dL	g/dL
Ref. ranges		(70-308)	(59-166)	(232-632)	(0.0-0.1)	(5.8-7.1)
Unoperated		181.2 ± 19.8	55.8 ± 9.2	175.2 ± 20.3	0.17 ± 0.10	5.7 ± 0.1
WZ42 pin	2 weeks	155.8 ± 26.7	57.3 ± 7.9	148.3 ± 13.6	0.18 ± 0.05	6.3 ± 0.3
Ti6Al4V pin	2 weeks	322.0 ± 94.4	66.3 ± 20.3	151.2 ± 21.8	0.14 ± 0.05	6.2 ± 0.1
WZ42 pin	8 weeks	294.5 ± 205.0	80.4 ± 13.9	163.2 ± 30.4	0.24 ± 0.05	6.4 ± 0.3
Ti6Al4V pin	8 weeks	204.8 ± 75.9	54.5 ± 17.9	201.5 ± 40.2	0.18 ± 0.05	6.4 ± 0.3
WZ42 pin	14 weeks	177.8 ± 47.1	65.6 ± 8.7	183.8 ± 33.3	0.20 ± 0.00	6.4 ± 0.1
Ti6Al4V pin	14 weeks	229.5 ± 198.5	56.8 ± 15.8	187.0 ± 33.5	0.18 ± 0.04	6.3 ± 0.3
WZ42 cuff	14 weeks	123.6 ± 36.3	64.0 ± 7.7	155.2 ± 21.7	0.20 ± 0.00	6.3 ± 0.3
Ti6Al4V cuff	14 weeks	154.4 ± 53.6	50.2 ± 3.4	163.6 ± 30.3	0.18 ± 0.04	6.2 ± 0.3

Name	Albumin	Urea N	Creatinine	Globulin	A/G Ratio
Units	g/dL	mg/dL	mg/dL	g/DI	
Ref. ranges	(3.2-3.7)	(13-19)	(0.3-0.5)	(2.6-3.5)	
Unoperated	3.3 ± 0.1	20.7 ± 1.9	0.37 ± 0.08	2.4 ± 0.1	1.4 ± 0.1
WZ42 pin	3.3 ± 0.2	20.3 ± 5.6	0.50 ± 0.00	3.0 ± 0.1	1.1 ± 0.1
Ti6Al4V pin	3.3 ± 0.1	17.6 ± 2.1	0.42 ± 0.04	2.9 ± 0.2	1.1 ± 0.1
WZ42 pin	3.5 ± 0.1	23.6 ± 2.1	0.52 ± 0.08	2.9 ± 0.3	1.2 ± 0.1
Ti6Al4V pin	3.7 ± 0.2	21.8 ± 2.9	0.53 ± 0.05	2.7 ± 0.1	1.3 ± 0.1
WZ42 pin	3.6 ± 0.1	22.2 ± 1.6	0.56 ± 0.05	2.7 ± 0.1	1.3 ± 0.1
Ti6Al4V pin	3.7 ± 0.2	21.8 ± 2.5	0.52 ± 0.04	2.6 ± 0.2	1.4 ± 0.1
WZ42 cuff	3.7 ± 0.2	24.8 ± 2.2	0.58 ± 0.04	2.6 ± 0.2	1.4 ± 0.1
Ti6Al4V cuff	3.6 ± 0.1	19.6 ± 1.9	0.52 ± 0.04	2.6 ± 0.2	1.4 ± 0.1

[0072] Similarly, serum biochemical parameters are shown in Table 3 above, with kidney function measured by creatinine and urea levels, and liver function measured by the albumin, alkaline phosphatase, bilirubin, and glucose. All the parameters measured remained within the reference ranges or near pre-operation levels, demonstrating little effect of the implanted alloy materials on the kidney and liver function as well as the metabolism.

[0073] Electrolyte parameters calcium, sodium, chloride, phosphorous, and magnesium were measured from the serum samples which are shown in Table 4.

TABLE 4

Average values of electrolyte parameters of naïve animals and animals implanted with WZ42 and Ti6Al4V alloy pins and cuffs at 2, 8, and 14 weeks after implantation.						
Name	Implantation time	Calcium	Sodium	Chloride	Phosphorous	Magnesium
Units		mg/dL	mmol/L	mmol/L	mg/dL	mg/dL
Ref. ranges		(9.5-13.9)	(146-151)	(98-104)	(5.6-16.8)	(3.8-5.5)
Naïve		9.8 ± 0.2	138.2 ± 1.9	100.5 ± 1.0	5.5 ± 0.2	2.0 ± 0.2
WZ42 pin	2 weeks	11.2 ± 0.1	144.8 ± 1.7	101.0 ± 1.4	8.5 ± 0.9	2.9 ± 0.3
Ti6Al4V pin	2 weeks	11.5 ± 0.5	143.8 ± 1.6	100.2 ± 2.2	9.7 ± 1.9	3.4 ± 0.4
WZ42 pin	8 weeks	11.6 ± 0.5	144.4 ± 1.9	100.6 ± 2.8	9.7 ± 1.4	3.5 ± 0.5
Ti6Al4V pin	8 weeks	11.8 ± 0.6	146.5 ± 1.0	100.0 ± 1.4	11.3 ± 0.8	3.9 ± 0.4
WZ42 pin	14 weeks	11.4 ± 0.3	147.8 ± 1.9	100.2 ± 2.0	9.8 ± 0.7	3.6 ± 0.2
Ti6Al4V pin	14 weeks	12.2 ± 1.1	145.2 ± 2.6	99.0 ± 2.0	9.6 ± 1.2	3.6 ± 0.6
WZ42 cuff	14 weeks	11.3 ± 0.2	147.0 ± 0.7	101.4 ± 0.9	9.1 ± 1.4	3.2 ± 0.2
Ti6Al4V cuff	14 weeks	11.6 ± 0.3	147.6 ± 1.1	99.6 ± 1.7	9.2 ± 0.7	3.3 ± 0.2

[0074] Magnesium levels remained in the low end of the reference ranges indicating no accumulation of degrading Mg from the implants in the collected blood. All the other electrolytes similarly remained consistent with levels of naïve rats and the prescribed allowable reference ranges.

[0075] ICP-OES results of the acid-digested liver and kidney demonstrated no accumulation of Mg exceeding the normal levels seen in the naïve control rats in the collected liver and kidney tissue in the WZ42 or Ti6Al4V groups. Ca and Zn concentration in the liver and kidney also did not deviate from the normal levels. Some differences were observed between the various groups, however, no significantly higher levels in the WZ42 groups of Mg, Ca, or Zn compared to naïve controls were observed. The concentration of other alloying elements (Y and Zr) measured from the digested liver and kidney were also perceived to be too low to be differentiated from normal levels, with Y being present in <0.7 µg/g dry mass in both liver and kidney, and Zr present in <2.2 µg dry mass in both liver and kidney.

Histological Examination of Liver and Kidneys

[0076] Conventional light microscopy images (FIG. 2 for kidney and FIG. 3 for liver) revealed that the cellular structure of the liver and kidney did not undergo any noticeable morphological changes or infiltration by inflammatory cells. No signs of obvious abnormalities were also observed in any of the sections of the organs.

In Vivo Corrosion of Mg-Y-Zn-Zr-Ca Alloy Pins and Morphology of Surrounding Bone

[0077] Representative cross-sectional micro-CT slices obtained from the femur-implant complex are shown in FIG. 4.

[0078] After two weeks of implantation, all the implanted pins had broken as seen in FIG. 4a, despite all pins appear-

ing to be intact after 1 week as observed by X-ray (FIG. 1). These pin failures occurred near the site of the femoral fracture, resulting in mal-union. In addition, sites of pits of corrosion appeared at the junctions where the pins were clamped in collets during lathe machining as seen in FIG. 4a. Both of these two regions where the corrosion/failure occurred corresponded with regions of presence of likely higher stress. Progressive degradation throughout the pins was observed at 8 and 14 weeks (FIG. 4b and c). Regions of the pin surrounded by the cortical bone appeared to degrade more slowly. Micro-CT scans of intact femurs with WZ42

wire cuffs wrapped around the midsection of the diaphysis (FIG. 4d) revealed what appeared to be new bone formation in the region surrounding the degrading cuffs indicated by arrows, despite the cuffs having completely degraded after 14 weeks when the scans were performed.

[0079] Following segmentation of the remaining WZ42 pins from the surrounding degraded product and bone, 3D reconstructions of the pins were created from which the volume was calculated. This remaining volume was used to calculate the corrosion rate at the end of 2, 8, and 14 weeks as shown in FIG. 5.

[0080] Degradation was found to occur more rapidly initially at 2 weeks, after which the corrosion rate was reduced and stabilized as seen by the lower corrosion rates calculated for 8 and 14 weeks. After the final time point of 14 weeks, approximately 43% of the original alloy pin volume remained.

Local Tissue Response to Mg-Y-Zn-Zr-Ca Alloy Pin

[0081] Femur explants were collected after 2, 8, and 14 weeks to assess the local tissue response to the WZ42 pins and cuffs and observe fracture healing. Sections of the bone from femurs containing the pins were stained using the Goldner's Trichrome method and are shown in FIG. 6.

[0082] After 2 weeks, in rats implanted with the WZ42 alloy intramedullary pins, dead spaces were observed over the fracture site in the fibrous tissue ("soft callus") that had formed around the bone. This was likely due to accumulation of hydrogen gas forming gas pockets (GP) from the degrading magnesium alloy as this was not observed in rats implanted with the Ti6Al4V pins. Osteoids (Od) had formed near the osteotomy region with fibrous tissue (Ft) surrounding the fracture site. After 8 weeks, the empty pocket over the fracture site was not perceived to be as prominent, potentially due to a slowing of the corrosion rate as mea-

sured by micro-CT (FIG. 5), dissipation of gas, and ingrowth of fibrous tissue. A greater presence of osteoids as well as new bone formation (Nb) in the periosteal region was observed progressively at 8 and 14 weeks. At 14 weeks' post-implantation, the fracture was not completely healed when fixed with either WZ42 or Ti6Al4V pins with narrow gaps remaining between the fragments of cortical bone not yet filled by mature cortical bone.

[0083] Alkaline phosphatase staining was conducted to observe osteoblast activity and the process of new bone formation in the region surrounding the defect. Osteoblast activity was more abundant surrounding the fracture in the femurs containing WZ42 at 8 and 14 weeks (FIG. 7c, e) compared to the femurs containing Ti6Al4V (FIG. 7d, f). The presence of osteoblasts appeared to peak at 8 weeks for the WZ42 group.

[0084] Goldner's Trichrome stained sections of tissue near the site of wire cuff implantation (FIG. 8) displayed new bone as seen in light blue-green as well as fibrous tissue in the region surrounding the Mg alloy cuff implant (FIG. 8a). In contrast, new bone formation was not seen around the inert Ti6Al4V cuff (FIG. 8b).

Discussion

[0085] The example described herein illustrates the response of WZ42 magnesium alloy when tested in a challenging orthopedic bone defect model prone to considerable stress corrosion, while at the same time demonstrating its biocompatibility without eliciting any toxicity. Moreover, the WZ42 alloy induced new bone formation and bone healing surrounding a fractured femur. The main animal model was a closed femoral fracture stabilized by an intramedullary pin. An evaluation of permanent metal pins for bone healing and mineralization was conducted. Despite these instances of permanent metals, such as stainless steel or Ni-Ti alloys being used to fix full osteotomies in rats, such an aggressive model representing the existence of and exposure to large dynamic stresses that will likely accelerate corrosion, has not been tested with magnesium alloys particularly, with the aim of assessing the toxicity and biocompatibility. The example also confirmed the safety of Mg alloy in this challenging model and analyzed the degradation behavior as a result of the high stress being placed on the Mg pins.

[0086] To assess the safety of the WZ42 implants, biochemical analysis of the blood and serum was conducted. The lower than expected platelet levels for the WZ42 and Ti6Al4V pin groups at 14 weeks, and WZ42 wire cuff group also at 14 weeks, was likely due to platelet clumping in samples, which was reported in many of the samples analyzed. The slightly elevated levels of white blood cells 2 weeks after surgeries for WZ42, as well as Ti6Al4V, represents a common post-surgical inflammatory response known to occur during wound healing, which returned to normal levels in a follow-up evaluation. This was paralleled by no clinical sign of any infection at the surgical site. The consistent electrolyte levels as measured in blood and the stable Mg concentration measured in the digested kidney and liver signifies that the degradation of WZ42 did not cause any disturbances in the balance of physiological electrolyte levels. Along with the unaltered serum biochemical parameters, it was suggested that liver and kidney functions were not affected by the WZ42 alloy degradation, concentrations of Mg, Ca, and Zn (elements contained in the

WZ42 alloy) in the liver and kidney did not rise above levels measured in naïve rats. Concentrations were also consistent with rats implanted with Ti6Al4V samples compared at the same time points.

[0087] To further demonstrate systemic biocompatibility, H&E staining of liver and kidney samples did not reveal any signs of organ alteration or damage. No focal mineralization, acute inflammatory cell infiltration, or necrosis were observed in kidney tissues. In the liver, no aggregates of inflammatory cells or features of hepatocellular necrosis such as irregular patchy areas of coagulation necrosis were observed. These results suggest that the WZ42 alloy and its degradation products are systemically biocompatible.

[0088] Progressive degradation was observed in the intramedullary WZ42 pins as seen in the reducing cross sectional area of the implants seen in FIG. 4(a-c), and calculated corrosion rate and volume loss shown in FIG. 5. Degradation appeared to occur preferentially at the fracture site, perpendicular to the fracture, where the stresses acting on the implanted pin are expected to be the highest. This synergy of mechanical loading combined with the corrosive environment of surrounding fluids in the body has been shown to cause sudden fracture of implants via the stress corrosion cracking (SCC) mechanism. This embrittlement phenomenon may occur even when the applied stress does not exceed the yield strength of the material, reducing the time to fracture and causing premature brittle failure. Magnesium, suffering from pitting corrosion, a source from which SCC can develop, has shown susceptibility to SCC in chloride solutions and simulated body fluids. Other localized regions of corrosion, such as near the end of the pin in FIG. 4a, occurred due to pre-existing flaws imparted during lathe machining, which increased the susceptibility to SCC. Degradation at the site of fracture was also promoted by the higher exposure to the surrounding fluid electrolyte due to small gaps between the two sides of the femur, acting to produce fluid shear stress and remove local OH⁻ ions to reduce the protection that arises from the passivation layer. During the early stages of healing after implantation when inflammatory responses were occurring, the characteristic hypoxic and acidic environment optimal for activities of polymorphonuclear leukocytes and tissue macrophages also resulted in higher corrosion rate due to the instability of magnesium hydroxide in acidic conditions and infiltration of these cells at the site of fracture causing phagocytosis of the metal debris. After this initial inflammatory phase and more rapid corrosion rate at 2 weeks, corrosion slowed when measured after 8 weeks as the surface of the Mg implant became further passivated and the fracture site enclosed in fibrous tissue, soft callus, and eventually newly formed bone. During the bone repair progression, the pH had risen ultimately becoming slightly alkaline to optimize alkaline phosphatase activity to perform its role in callus mineralization, thereby becoming more conducive to the formation of the passivating magnesium hydroxide layer on the surface of the degrading WZ42 pins. The percentage volume remaining did not significantly change between 2 and 8 weeks because the measurements were taken from different samples at each time point. For the implants used to calculate the 8 week measurements, the corrosion rate was lower such that over time, the volume remaining was not significantly different from the samples measured after 2 weeks, which degraded at a much faster rate. The variation between samples that caused the difference in degradation for 2

versus 8 week samples may be due to variability in when the pins failed, or pins not being fully surrounded by cortical bone due to mal-union and thus, being exposed to more surrounding fluid leading to enhanced corrosion. The thin WZ42 wires wrapped around the outside of the femur, while being visually apparent in X-ray after 1 week (FIG. 1d), were completely degraded after 14 weeks as seen in FIG. 4d as a result of their smaller profile as well as being exposed to a much more corrosive environment having been placed on the surface of the femur instead of being surrounded by the cortical bone.

[0089] The effect of the degrading Mg alloy on the surrounding tissue was investigated via Goldner's Trichrome and ALP staining after 2, 8, and 14 weeks' post-implantation of the WZ42 and Ti6Al4V intramedullary pins and extra-cortical cuffs. After 2 weeks in rats implanted with the WZ42 alloy (FIG. 1a, FIG. 6a), gas pockets (GP) were observed over the fracture site forming empty cavities in the surrounding in fibrous tissue. This was likely due to accumulation of hydrogen gas from the degrading magnesium alloy, as this is not observed in the Ti6Al4V pins. Despite potential concerns of the effects of these gas pockets on the healing processes, no bone erosion due to these cavities was observed in rabbits after 1 year follow-up assessment of another Mg alloy also containing Mg, Y, and Zr similar in composition to the WZ42 alloy used herein. After 8 weeks (FIG. 6c), the gas pocket over the fracture site fixed with WZ42 was not as prominent, potentially due to slowing of corrosion rate, absorption of gas, and ingrowth of fibrous tissue. Clinical implantation of Mg alloy screws also revealed hydrogen gas formation soon after implantation, which disappeared through absorption into surrounding tissue by approximately 2-4 weeks post-surgery. Despite the appearance of hydrogen gas, formation of calcification matrix was not inhibited to initiate the bone formation process, allowing for success in the long-term clinical study.

[0090] The bone healing process of the fractured rat femurs consisted of several phases as observed using staining at various time points. After two weeks, the inflammatory phase of fracture healing appeared to have passed, with bone healing entering the reparative phase characterized by the development of callus tissue forming in and around the fracture site to be later replaced by bone. The presence of osteoids (Od) was observed near the bone with fibrous tissue (Ft) to indicate the initial composition of the soft callus surrounding the fracture site. A greater presence of osteoids, as well as new bone formation (Nb) in the periosteal region, was observed progressively after 8 and 14 weeks. After 14 weeks, the fracture was not yet completely healed with full woven bone when fixed with either WZ42 (FIG. 6e) or Ti6Al4V (FIG. 6f) pins. However, the presence of mineralized tissue indicated callus calcification as the mineralization process progressed. The elevated new bone formation seen in the WZ42 group at 8 and 14 weeks, which was further confirmed by ALP staining (FIG. 7). ALP is necessary for mineralization of the callus providing phosphate ions for precipitation with calcium to form the apatite mineral phase. Osteoblast activity as indicated by ALP staining demonstrated promotion of new bone formation in the region surrounding the defect at the leading edge to heal the fracture. ALP activity peaked at 8 weeks for the WZ42 group with higher activity compared to the Ti6Al4V group. Despite the fracture not having healed fully due to the instability of the intramedullary fixation, the healing process

appeared to be un-encumbered. In fact, the prevalence of new bone formation as seen in the mineralized new bone and osteoblast activity in regions adjacent to the Mg alloy implants, confirmed results of studies reporting enhanced new bone formation around Mg-based implants related to the cellular activity of Mg such as osteoconductivity of the phosphate layer forming on the surface of magnesium alloy-based implants, and promotion of enhanced mineralization from bone marrow stromal cells. Additionally, the consistent observations of a normal healing response of a fibrous capsule enclosing the operation site with no abnormal presence of inflammatory cells at the implant site, has been observed. Mg scaffolds show good biocompatibility that is indicative of the local biosafety of the Mg alloy. Without a defect created in the case of the wire cuff placed over the cortical bone, the phenomenon of enhanced new bone formation was confirmed in the region surrounding the Mg alloy cuff implant compared to the inert Ti6Al4V (FIG. 8).

[0091] Overall, the positive biocompatibility and signs of healing with new bone formation observed suggest that the WZ42 alloy is a suitable candidate for orthopedic applications provided care is exhibited to limit the mechanical stresses placed on the implant, and that a consistent finish on the alloy is obtained by careful machining in order to reduce the onset of rapid corrosion and potential failure brought on by the phenomenon of stress corrosion cracking. Immobilization of the fracture following implantation of the pins by use of external supports can serve to alleviate the direct exposure of the implants to the extreme dynamic stresses leading to accelerated corrosion. The model tested here provided an ideal environment contributing to creating a high dynamic stress on the implant site. Thus, loading the femur and completely transferring this load directly onto the Mg intramedullary pin leading to a highly aggressive load and corrosion condition causing the pins to ultimately fail. The dynamic nature of the stresses combined with aggressive movement fostered by ambulation of the animals lead to variation in the progression of the corrosion. As a result, variability in the non-union and healing could be seen (FIG. 4a, 4b). This aggressive loading bone model showed that, despite having such an aggressive condition around the implanted scaffold that could be perceived as an extreme event along with accelerated corrosion, local and systemic biocompatibility of the alloy was still observed. Despite the initial higher corrosion rate, hydrogen gas formation was however, fairly limited and not externally noticeable, while the surrounding tissue response, including kidney and liver, and blood parameters all remained normal. Thus, these observations allude to the biocompatibility and safety of these alloys. With temporary unloading and immobilization, as is the standard of treatment for orthopedic injuries, the risk of failure of the WZ42 alloy would likely be diminished, still rendering the alloy as a suitable orthopedic implant material, with potential success in other medical device applications. Semi or non-load bearing environments placed on orthopedic Mg implants demonstrate the safety and efficacy of the implants. The model implemented without any harness demonstrates the safety and non-toxicity of Mg, and the alloying elements used to process the WZ42 alloy to the extent of the size and dimensions used compared to the rats employed.

Conclusions

[0092] WZ42 (Mg-Y-Zn-Zr-Ca) alloy pins were implanted into the intramedullary cavity of fractured rat femurs and as wires wrapped around the midsection of un-altered femurs, comparing the Mg alloy to Ti6Al4V. Degradation of the intramedullary pins led to failure due to perceived stress related corrosion initiated at the osteotomy site of high mechanical loading and surrounding vasculature aiding corrosion. However, the WZ42 alloy was found to be biocompatible with no recognizable accumulation of Mg or alloying elements in the blood, liver, or kidney, and no adverse effects on blood count, or metabolic, kidney, and liver function. Histology of the local area at the implant site showed normal fracture healing and new bone formation. These positive results, despite the challenging nature of the model, indicated the suitability of this alloy, WZ42, for orthopedic fixation applications.

Example 2

[0093] This example evaluates the degradation effects, local tissue response, and systemic toxicity of Mg-4Zn-0.1Sr-0.5Zr pins implanted in the intramedullary region of fractured rat femurs. Mg alloys are not intended for use as femoral rods. However, a rat femoral fracture model was selected to primarily assess the degradation profile of Mg-Zn pins and demonstrate the toxicity of Mg-Zn pins under load-bearing conditions.

Materials and Methods

Alloy Processing and Femoral Pin Fabrication

[0094] Mg-4Zn-0.1Sr-0.5Zr (Mg-Zn) alloy was synthesized using an electrical resistance furnace (Wenesco Inc.). Pure Mg (US magnesium Inc. 99.97%), Zn shots (Alfa-Aesar 99.99%), and Mg-30Sr master alloy were melted in a mild steel crucible. The total melt amount was 250 g. The melting process was performed using 0.5% SF6 plus balance Ar protective gas atmosphere to protect the molten magnesium alloy from oxygen. The molten mixture of Mg, Zn, and Sr was homogenized at 700° C. and the zirconium content was added using Zirmax® (Mg-33.3 wt % Zr) master alloy (Magnesium Elektron Ltd.). After 1 and 5 minutes, the liquid melt was further homogenized by stirring for 10 seconds to dissolve and disperse the zirconium particles uniformly. The melt was maintained at 700° C. for 30 minutes and following, the molten liquid was poured into a mild steel mold (44.5 mm×82.5 mm) preheated at 500° C.

[0095] The middle part of the as-cast Mg-Zn-Sr-Zr (Mg-Zn) alloy following removal of the top, sides and the bottom, was machined to a dimension of 37.6 mm diameter and 60 mm height by using a lathe. The as-cast Mg-Zn-Sr-Zr (Mg-Zn) alloy was then heat-treated at 300° C. for 1 hour, quenched in oil, and annealed at 205° C. for 12 hours. Following heat treatment, hot extrusion was performed using an extrusion ratio of 30:1 at North Carolina A&T University (Greensboro, NC).

Animal Study Design

[0096] An animal study was conducted in accordance with a protocol approved by Animal Care and Use Committee (IACUC) at the University of Pittsburgh. Groups, time points, and number of animals involved in the current chapter are identified in Table 1A.

TABLE 1A

Groups, time points, and number of animals used for Ti and Mg—Zn device implantation.		
Group	Time point	N/time point
Ti alloy pins	2 and 14 weeks	5
Mg—Zn pins	2 and 14 weeks	5
Ti alloy cuffs	14 weeks	5
Mg—Zn cuffs	14 weeks	5

[0097] Thirty, Sprague-Dawley rats of approximately 250 g of body weight were used. Fifteen rats were randomly selected for Ti alloy implantation and the other rats were implanted with Mg alloy pins. For each implant material, ten rats were implanted with pins for 2 and 14 weeks and five rats were implanted with the cuff for 14 weeks. For each pin implanted, the right femur of each rat was approached laterally, and an osteotomy was performed in the middle of the femur using a dremel drill with a diamond wheel blade. A pin of Ti or Mg alloy was inserted into the intramedullary region to achieve a stable reunion of the fractured femur. Five rats from both Ti and Mg groups were sacrificed for fracture healing and toxicity analysis after 2 or 14 weeks of implantation. For each cuff implantation, a cuff of Ti or Mg-Zn alloy was implanted around the unfractured femur for toxicity analysis after 14 weeks.

Radiographic Imaging and Computer Tomography Analysis

[0098] X-ray images of all animals were obtained after 7 days to observe implant location and alignment of fractured femurs. The Mg-Zn alloy pins before implantation were scanned using micro-computed tomography (microCT) (VivaCT40; Scanco Medical, Switzerland). The harvested femurs were also scanned using microCT, after embedding in plastic. Analysis of the microCT images were performed using Mimics (Materialise, Belgium) to calculate the degradation rate of the Mg alloy pins and assess fracture healing. Averages and standard deviations of five sample measurements were reported and t-test was used to determine any significant mean differences with a p-value less than 0.05 for Mg-Zn pin groups at different time points.

Blood Test

[0099] Blood samples were obtained before operation and after euthanasia at 2 and 14 weeks. The samples collected in K2-EDTA were sent to Marchfield Labs (Cleveland, OH) for hematologic analysis. Red blood cell count, hemoglobin, hematocrit, platelet count, and white blood cell count were analyzed using a Sysmex XT2000i Automated Hematology Analyzer (Sysmex Corporation, Japan). For biochemical analysis, the blood samples were maintained at room temperature to clot for 30 minutes and centrifuged at 2,000 rpm for 10 minutes. The supernatant serum samples were analyzed using an Olympus AU chemistry analyzer (Olympus Corporation, Japan). Alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, total and direct bilirubin, cholesterol, glucose, urea, creatinine, phosphorus, chloride, potassium, sodium, and magnesium were accordingly measured. Averages of three sample measurements were reported and compared to the reference ranges for each parameter.

ICP Analysis

[0100] Harvested liver and kidney tissues were dried in an oven at 70° C. overnight. Dried tissue samples were then ground using a mortar and pestle. 0.5 g of ground tissues were dissolved in 5 mL of concentrated nitric acid that was kept heated at 130° C. for 14 hours, and supplemented with 1 mL of 30% hydrogen peroxide. Sample solutions were then diluted 10 times and measured using an inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP duo 6500 Thermo Fisher, Waltham, MA), with standard solutions of the various elements being analyzed. Averages and standard deviations of three sample measurements were reported and one-way ANOVA was used to determine any significant mean differences with a p-value less than 0.05 for all other groups.

Soft Tissue Histology

[0101] Harvested liver and kidney tissues were fixed in 10% neutral buffered formalin for 48 hours. The fixed tissues were sectioned in small pieces, dehydrated in ethyl alcohol series from 70% to 100%, cleaned using xylene substitute and embedded in paraffin. Paraffin tissue blocks were then sectioned using a rotary microtome. Tissue slices were accordingly dewrinkled on a warm water bath and transferred to glass slides. After drying, tissue slides were imaged using an optical microscope after staining with hematoxylin and eosin (dyes) and mounted using a mounting solution.

Bone Tissue Histology

[0102] Undecalcified embedding was used to perform histology analysis of the harvested femurs with implants. Harvested femurs were fixed in 70% ethyl alcohol for 72 hours. The fixed femurs were then dehydrated in diluted ethyl alcohol from 70% dilution to 100% consecutively. The femurs were cleaned in xylene and embedded in poly methyl methacrylate (PMMA) (OsteoBed, Life Technology). Then, 7 to 10 μ m tissue sections were obtained from the embedded femurs using a rotary microtome with a tungsten carbide blade. Sections were adhered to tape to prevent shattering during sectioning. Sections were subjected to Goldner's Masson Trichrome and alkaline phosphatase (ALP) staining. The stained sections were mounted on a glass slide using a glycerol solution and observed under an optical microscope.

Results

In Vivo Degradation of the Magnesium-Zinc-Zirconium-Strontium (Mg-Zn-Zr-Sr) Alloy Pins

[0103] X-ray radiograph images of Ti and Mg-Zn pin-implanted rats after one week of osteotomy surgery, as

shown in FIG. 9, were obtained to identify the position of Mg-Zn and Ti pins and fracture fixation stability. In the x-ray image, some visible hydrogen gas evolution was observed around the Mg-Zn implants as shown in FIGS. 9b and 9c. Rats implanted with both Ti and Mg-Zn pins exhibited normal movement and ambulatory behavior.

[0104] Harvested femurs exhibited normal fracture healing response with intramedullary pins. Callus formation was observed around the wound. Callus formed on the femurs with both Ti and Mg-Zn pins exhibited no visible difference. However, the callus formed on the femurs after 8 and 12 weeks of implantation was more hardened compared to the callus after 2 weeks of implantation. The fracture femurs with Mg-Zn pins was not aligned as straight as the femurs with Ti pins.

[0105] FIG. 10 shows the representative micro-computed tomography (micro-CT) images of rat femurs with Ti or Mg-Zn pins after plastic embedding. A femur with a broken Mg-Zn pin resulted in a misalignment of fractured bones. At 2 weeks, three out of five Mg-Zn pins were fractured into two pieces. The Ti pins, however, exhibited no fracture or damage due to the load. Mal-union of the femurs with broken Mg-Zn pins can lead to a significant difference in the fracture healing. The micro-CT images at 14 months for both Ti and Mg-Zn pins implanted, as shown in FIG. 3, indicated that the fracture healing process was not completed. More hydrogen gas bubbles were evolved around Mg-Zn pins following 2 weeks of implantation not distinguishable in micro-CT but was noticeable in the histology (later discussed herein). After 14 weeks, the gas bubbles were not distinguishable in the micro-CT images.

[0106] The remaining volume of Mg-Zn pins in the intramedullary region was analyzed from the micro-CT images. The remaining volume of Mg-Zn pins after 2 weeks of implantation was 87.7%. After 14 weeks, the remaining volume was significantly decreased to 42.0%. Corrosion rates of Mg-Zn pins were calculated from the volume loss and original surface area. Mg-Zn pins for 2 weeks of implantation exhibited the corrosion rate of 0.91 ± 0.65 mmpy. The corrosion rate at 2 weeks was anticipated to be higher than the other time points since the Mg-Zn pins was exposed to the largest mechanical stress at the 2 weeks' time point. The Mg-Zn pins for 14 weeks of implantation continued to degrade with larger surface area being exposed which resulted in the corrosion rate of 0.77 ± 0.30 mmpy.

Blood Test Results

[0107] Table 2A summarizes blood test results of rats before and after osteotomy surgeries for 2 and 14 weeks of Ti and Mg-Zn pins/cuffs implantation.

TABLE 2A

Hematologic analysis results from blood panel test after Ti pin (2 and 14 weeks), Mg—Zn pin (2 and 14 weeks), and Mg—Zn cuff (2 weeks) implantations.					
Name	Implantation time	Red Blood Cell Count	Hemoglobin	Platelet Count	White Blood Cell Count
Units		$10^6/\mu\text{L}$	g/dL	$10^3/\mu\text{L}$	$10^3/\mu\text{L}$
Ref. ranges		(7.00-9.00)	(13.7-16.8)	(680-1280)	(1.1-7.5)
Pre-operation		7.4	14.1	618.3	6.8
Ti pin	2 weeks	7.8	14.9	656.0	9.0
Mg—Zn pin	2 weeks	7.4	14.1	547.0	3.7

TABLE 2A-continued

Hematologic analysis results from blood panel test after Ti pin (2 and 14 weeks), Mg—Zn pin (2 and 14 weeks), and Mg—Zn cuff (2 weeks) implantations.					
Name	Implantation time	Red Blood Cell Count	Hemoglobin	Platelet Count	White Blood Cell Count
Ti pin	14 weeks	7.5	14.0	563.0	5.9
Mg—Zn pin	14 weeks	7.3	13.7	598.0	4.5
Ti cuff	14 weeks	7.6	13.8	637.0	5.8
Mg—Zn cuff	14 weeks	7.2	13.4	537.0	6.2

[0108] Red blood cell, hemoglobin, and platelet count exhibited no significant difference in the groups for the different implants and the different time durations. White blood cell count after 2 weeks of Ti pin implantation was significantly higher compared to the Mg-Zn group. However, the white blood cell count still remained within a reference range.

[0109] Biochemical analysis results rats before and after osteotomy surgeries for 2 and 14 weeks of Ti and Mg-Zn pins/cuffs implantation are listed in Table 3A.

[0110] ALT after 2 weeks of implantation for both Ti and Mg-Zn pins exhibited significantly higher level compared to the implants for the other time points. However, these ALT values still remain within a reference range. For ALP, TBIL, TP, ALB, UA, CR, and GLB, all groups exhibited no significant difference between implants and implantation duration suggesting no signs of liver or kidney damage due to degradation of Mg-Zn pins.

TABLE 3A

Biochemical analysis on blood serum after Ti pin (2 and 14 weeks), Mg—Zn pin (2 and 14 weeks), and Mg—Zn cuff (2 weeks) implantations.										
Name	Implantation time	Glucose	ALT (GPT)	ALP	Total Bilirubin	Total Protein	Albumin	Urea N	Creatinine	A/G Ratio
Units		mg/dL	U/L	U/L	mg/dL	g/dL	g/dL	mg/dL	mg/dL	g/DI
Ref. ranges		(70-308)	(59-166)	(232-632)	(0.0-0.1)	(5.8-7.1)	(3.2-3.7)	(13-19)	(0.3-0.5)	(2.6-3.5)
Unoperated		181.2	55.8	175.2	0.17	5.7	3.3	20.7	0.37	2.4
Ti pin	2 weeks	322.0	132.6	151.2	0.14	6.2	3.3	17.6	0.42	2.9
Mg—Zn pin	2 weeks	293.0	123.0	140.0	0.18	6.7	3.5	20.5	0.53	3.2
Ti pin	14 weeks	229.5	56.8	187.0	0.18	6.3	3.7	21.8	0.52	2.6
Mg—Zn pin	14 weeks	124.2	57.8	181.8	0.20	6.3	3.6	22.2	0.56	2.7
Ti cuff	14 weeks	154.4	50.2	163.6	0.18	6.2	3.6	19.6	0.52	2.6
Mg—Zn cuff	14 weeks	203.0	59.4	190.4	0.16	6.5	3.8	23.2	0.52	2.7

[0111] Calcium, sodium, chloride, phosphorous, and magnesium ion levels in the serum before and after implantation are listed in Table 4A. No significant difference in these ion levels were found between implants or implantation duration. All values remained within the reference range suggesting that there were no changes to the systemic ion concentration due to implantation and the consequent degradation of the Mg-Zn-Zr-Sr alloy pins.

TABLE 4A

Electrolyte levels of blood serum after Ti pin (2 and 14 weeks), Mg—Zn pin (2 and 14 weeks), and Mg—Zn cuff (2 weeks) implantations.						
Name	Implantation time	Calcium	Sodium	Chloride	Phosphorous	Magnesium
Units		mg/dL	mmol/L	mmol/L	mg/dL	mg/dL
Ref. ranges		(9.5-13.9)	(145-151)	(98-104)	(5.6-16.8)	(3.8-5.5)
Unoperated		9.8	138.2	100.5	5.5	2.0
Ti pin	2 weeks	11.5	143.8	100.2	9.7	3.4
Mg—Zn pin	2 weeks	12.1	141.3	100.3	12.0	3.7
Ti pin	14 weeks	12.2	145.2	99.0	9.6	3.6
Mg—Zn pin	14 weeks	11.3	146.8	100.6	9.4	3.3
Ti cuff	14 weeks	11.6	147.6	99.6	9.2	3.3
Mg—Zn cuff	14 weeks	12.2	148.0	99.8	8.7	3.5

ICP Analysis on Liver and Kidney

[0112] ICP analysis of the liver or kidney was performed to examine any form of Mg accumulation in the organs after implantation of the Mg-Zn alloy pins and cuffs in comparison to the Ti control. Mg concentration determined in the kidney harvested from the experimental groups containing Mg-Zn implants exhibited no accumulation of Mg when compared to the concentration of kidney from the non-operated groups. Mg concentration observed in the liver tissue from the non-operated controls was in the range of 521 µg of Mg per gram of dried tissue. Regardless of implantation time, Mg-Zn alloy groups implanted with pin did not show any significant difference with the control level in Mg concentration of liver samples. The observation was consistent with blood test results indicating that implantation of the Mg-Zn alloy pins and cuffs in the rat femoral model exhibited no systemic toxicity.

H&E Staining of Liver and Kidney

[0113] Hematoxylin and eosin staining of liver and kidney tissue sections were performed to visualize any histological differences in tissue morphology due to Ti and Mg-Zn pin implantation. Liver sections of both Ti and Mg-Zn groups after 2 and 14 weeks of implantation, as shown in FIG. 11, exhibited a normal distribution of hepatocytes with clearly visible nuclei and central vein. In the kidney histology, no visible difference in glomeruli morphology, Bowman's space, capillaries, and convoluted tubules was observed between Ti control and Mg-Zn groups following 14 weeks of implantation. Histological morphology of liver and kidney tissues of all experimental groups displayed similar morphology as the non-operated control, and no difference was observed in between the groups or at different time points although data is not shown. In addition to ICP and blood test results, the H&E staining confirmed no damage to vital kidney and liver organs due to degradation following implantation of the biodegradable Mg-Zn pins. Although the H&E staining cannot be used to determine any accumulation of Mg, the fact that the histology showed normal and functional tissue was likely indicative of no accumulation related damage of the kidney and liver tissue, and potentially with time, there will be removal of Mg following the normal excretory process prevalent in the body.

Bone Tissue Histology

[0114] Goldner's Masson Tri chrome and alkaline phosphatase staining of rat femoral sections after plastic embedding revealed a typical fracture healing response as shown in FIGS. 12 and 13. After 2 weeks of implantation, the femurs with Ti and Mg-Zn alloy pins exhibited endochondral bone development and fibrous tissue formation around the fracture. Bone tissue section of the femur with Mg-Zn alloy pins after 2 weeks of implantation exhibited a gas pocket due to the degradation of Mg-Zn pins. After 14 weeks of implantation however, femurs with both Ti and Mg-Zn alloy pins exhibited bone remodeling and intramembraneous bone formation. In addition, the gas pocket of femurs with Mg-Zn pins was filled with fibrous tissues. Alkaline phosphatase activity (ALP) was observed around the fractured bones after 2 and 14 weeks of implantation. ALP was more present in the intramedullary region after 2 weeks. However, after 14 weeks, stained ALP signal was present along the fractured surface of the femurs, and stronger ALP activity was

shown in the image of ALP staining of femoral tissue with Mg-Zn alloy. Fracture repair of either Ti or Mg-Zn groups was not completed after 14 weeks of implantation.

Discussion

[0115] Biodegradable Mg alloys have gained considerable attention as discussed earlier for their potential to provide comparable or even improved benefits in fracture repair, and for bone fixation compared to biodegradable polymer and permanent bioinert metallic devices. Mg is characterized by mechanical properties being matched to natural human bone. Alloys are designed to exhibit mechanical properties with better match to natural bone while exhibiting the desired timely corrosion rates with more biocompatible degradation products, as compared to biodegradable polymers that tend to provide acidic by-products while also lacking the desired osteogenic potential to function as an acceptable bone scaffold system. The rapid corrosion of biodegradable Mg alloys can cause hydrogen gas evolution and immature mechanical failure, warranting the need for improved alloy design and other surface engineered strategies to control the corrosion rates while preserving the mechanical strengths. Hence, in vivo degradation and toxicity of Mg alloys have been widely studied to demonstrate the much desired biosafety and efficacy as candidate biomaterials for implantable medical devices. Orthopedic devices are often used in load-bearing conditions and metals often tend to corrode more rapidly when stress is applied via the well-known stress corrosion mechanisms. This is a common fixture and often the mode of much observed problems to date in permanent metal devices which also reveal stress induced corrosion, wear, and debris formation.

[0116] The examples tested degradation properties and biocompatibility of Mg-Zn-Sr-Zr alloys under load-bearing condition. Mg alloys are not intended for use as a femoral rod since they are known to degrade in the body. However, a femoral fracture model using intramedullary pins can exert a significant load on the implant material to cause a stress-induced corrosion for simultaneously examining relevant physiological response, such as bone healing, inflammatory response, and systemic toxicity. Thus, serving as an ideal bone model system to study the initial fracture healing response under the presence of stress. In addition to the fracture model, a group of rats were implanted with femoral cuffs for 14 weeks to compare any difference in systemic toxicity due to implantation at the femoral defect site.

[0117] In vivo degradation of Mg-Zn pins and cuffs was assessed using x-ray radiographs after 1 week of surgery. It is widely accepted that hydrogen gas bubble evolves in the earlier time points and tend to kinetically slowdown in 2-3 weeks. Mg alloys implanted in bone without a significant load also are reported to show slow degradation exhibiting no gas bubbles, while Mg alloys in subcutaneous region exhibit significant gas bubbles due to the surrounding vascularization and presence of blood flow. Both Mg-Zn alloy pins and cuffs were observed to be surrounded with hydrogen gas in the surrounding tissue. Bone histology results were also consistent with the x-ray images showing gas pockets near the fracture site in the image following 2 weeks of Mg-Zn pin implantation (see FIG. 9). Both x-ray radiograph and bone histology results indicated that the corrosion of Mg-Zn alloy implants in rat femurs were rapid enough to

create gas bubbles. However, the gas bubbles appeared to be eliminated after 14 weeks of implantation as shown in bone tissue histology images.

[0118] Computed tomography was used to perform fracture healing and determine quantitatively the degradation rate. Failure of the Mg-Zn alloy pins was observed in fractured femurs after 2 weeks of implantation. After 14 weeks, however, callus formation and bone remodeling around the fracture site were exhibited. Femurs with both Ti and Mg-Zn alloy pins revealed similar bone repair response. Rat femoral fracture models typically require up to 5 months to complete the fracture healing process. Three out of five rat femurs with implantation of Mg-Zn pins for 14 weeks were observed with a mal-union that might have resulted from the breakage of the pins due to the excessive stress experienced by the animals following immediate surgery and allowing the rats to ambulate subjecting the area to significant load serving as a classic stress corrosion fracture model. The bone histology and CT results showed acceptable and favorable bone healing responses, as indicated by the other femurs in union exhibiting better bone healing with more bone remodeling and united callus formation over the cortical bones (See FIGS. 10, 12, and 13). These results demonstrate the potential safety and efficacy of the Mg-Zn alloy system for orthopedic applications.

[0119] Mg-Zn alloys are known to degrade with higher corrosion rates compared to Mg alloys under non-load bearing condition. Based on these results, rapid corrosion of Mg-Zn alloy pins under load-bearing conditions did not significantly affect the fracture repair in terms of adverse local tissue response. However, mechanical strength of Mg alloys decreases with corrosion and therefore, the Mg-Zn alloy pins underwent failure which could have affected the fracture healing outcome negatively. Although, not negating the potential applicability of the system for orthopedic applications.

[0120] Systemic toxicity of rats with Mg-Zn alloy pins was assessed after 2 and 14 weeks of implantation, when 15% and 55% of total volume of the implants were resorbed. In addition, a group of the Mg-Zn alloy cuffs was assessed to evaluate the toxicity of Mg-Zn alloy in contact with both bone and muscle. After 14 weeks, the cuffs were fully resorbed. Blood and serum examination was focused on hematologic and biochemical analysis to detect any disruption in blood, liver and kidney tissue state. Blood cell count and biochemical parameters were maintained within the reference ranges, and there were no significant differences among Ti, Mg-Zn, and control groups. Recent publications also reported no significant abnormality in blood test results following in vivo degradation of Mg alloys. BUN, CR, and UA from serum analysis also indicates no significant changes in the renal function. No accumulation of Mg in liver and kidney was also found following inductively couple plasma analysis conducted on the digested tissues. Histology of liver and kidney also displayed histomorphology pattern, mirroring healthy tissue. Excess Mg in body is known to excrete in urine after renal filtration. Bodily Mg concentration beyond the tolerance limit can, however, cause renal failure. Based on the published literature, the toxicity test methods described herein are effective because high Mg dose in body does not cause local accumulation in a specific organ other than liver or kidney. Overall, the observations from blood test, Mg concentration, and histology results consistently indicated that the Mg-Zn alloy

implants and their degradation products are biocompatible for use as orthopedic implants under load-bearing conditions.

Conclusions

[0121] Mg-Zn alloy, in comparison to Ti alloy as a control, was examined as femoral pins under load-bearing conditions using a rat femoral fracture model. Localized stress on the Mg-Zn alloy pins caused stress corrosion. Hence, hydrogen gas pockets were observed around the fracture site initially, and some pins tended to lose their mechanical stability after the implantation for 2 weeks. However, normal bone healing was displayed following bone histological analysis. No fibrous capsule formation or adverse immune response was observed in local tissues around the Mg-Zn alloy implant devices as well. Furthermore, degradation of Mg-Zn implants caused no significant changes in hematologic or biochemical markers, assessed using blood panel tests. Magnesium concentration of liver and kidney demonstrated no accumulation of Mg in these organs, as well following elemental analysis of the tissue for the specific alloying elements. Histology of liver and kidney also displayed no organ damages due to the Mg-Zn alloy implants. Overall, the results suggest that Mg-Zn alloy demonstrates favorable biocompatibility under load-bearing conditions.

EXAMPLE 3

[0122] An alloy labeled as WJ41 having the following composition: 4% Y, 0.6% Sr, 0.4% Zr and a remainder of Mg, was subjected to melting and casting using the method described below.

[0123] Ingots of elemental magnesium (99.97% pure from U.S. Magnesium, Inc.), and magnesium-yttrium master alloy (30 wt. % yttrium) were weighed according to the nominal composition. The ingots were melted together in a graphite crucible (200 g batch) inside a quartz tube of a vacuum induction furnace to preclude oxidation of the pure elements. The graphite crucible preloaded with batch and the quartz tube assembly were purged with UHP argon several times and vacuumed subsequently to achieve a moisture-free environment prior to induction melting. The induction melting then was conducted and repeated several times in order to achieve compositional homogeneity. The initial alloy produced by the induction melting was cleaned thoroughly from any residue or oxide scale and re-melted subsequently in a mild steel crucible using an electrical resistance furnace (from Weneco, Inc.). The melting and pouring temperature were maintained at about 700° C., and once the temperature was reached, zirconium was added using Zirmax® (Mg-33.3% Zr) master alloy (from Magnesium Elektron, LTD.). The liquid melt was stirred for about 10 seconds after 1-minute and 5-minute intervals to dissolve and disperse the zirconium particles uniformly into the melt. Strontium was also added and melted. The melt was held for about 30 minutes at 700° C. and then poured onto a copper mold (1.5"×0.5") and a steel mold (2.0"×1.5") at room temperature. The as-cast samples were solution treated ("T4") at 525° C. for about 2 hours inside a tubular furnace covered with magnesium gettering powder under a protective atmosphere of argon and sulfur hexafluoride, and then quenched into water. Thin square plates (10×10×1 mm³) of samples were sectioned (using a Buehler Precision Saw Simplot 1000) from the as-cast and the T4 samples and were characterized

by X-ray diffraction (XRD) using Philips XPERT PRO system employing the $\text{CuK}\alpha$ ($\lambda=1.54056 \text{ \AA}$) radiation operated at 45 kV and 40 mA to determine the phase evolution and formation. The thin plate samples from the as-cast and T4 conditions were also used for electrochemical corrosion tests. Each square plate sample was mechanically grinded and polished to 2000 grit; ultrasonically cleaned in acetone, absolute ethanol and distilled water; and then dried in a vacuum oven at a temperature of 50° C.

[0124] The cast material demonstrated single phase Mg according to the X-ray diffraction pattern, without indication of the presence of secondary phases, which clearly confirmed the formation of single-phase solid solution.

[0125] However, microstructure analysis using optical microscopy and scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) revealed the presence of secondary phases containing Mg, Y, Zr and Mg, Al (an impurity), Sr, and Y.

[0126] Potentiodynamic corrosion tests were carried out using a scanning rate of 1 mV/s. A three-electrode cell was employed with platinum as the counter electrode, Ag/AgCl as the reference electrode, and the sample mounted in epoxy resin as the working electrode. The test was performed in Hank's Balanced Salt Solution (HBSS) and held at 37.4° C. Before each measurement, the sample was immersed in the corrosion media to provide stability. The cathodic and anodic portions of the generated Tafel plots were fit linearly to allow the calculation of corrosion potential, E_{corr} and the corrosion current density, i_{corr} , which was used to calculate corrosion rate according to ASTM G102-89, with the results as reported in Table 1C. The corrosion rate for the WJ41 cast alloy was slightly higher than the commercially obtained as-drawn pure Mg.

TABLE 1C

Electrochemical corrosion results of WJ41 alloy compared to pure Mg.			
	Corrosion potential (V)	Corrosion current density ($\mu\text{A}/\text{cm}^2$)	Corrosion rate (mm/yr)
WJ41 as-cast	-1.51 ± 0.02	6.60 ± 0.82	0.15 ± 0.02
Pure Mg as-drawn	-1.49 ± 0.01	4.38 ± 0.26	0.10 ± 0.01

[0127] The Mg-Y-Sr-Zr alloy (WJ41) after casting exhibited secondary phases containing Mg, Y, Zr and Mg, Al, Sr, and Y. The presence of these secondary phases may have resulted in the slightly increased corrosion rate compared to pure Mg. It is expected that extrusion would improve the corrosion rate of WJ41.

Example 4

[0128] The effect of adding Sr to Mg-Zn-Zr (designated as ZK40) was further demonstrated using the following results of Mg-Zn-Zr-Sr (designated as ZJK40 and ZJK41). A comparison of the mechanical, corrosion, and cytocompatibility properties were studied for Mg-Zn-Zr (ZK40) and Mg-Zn-Zr-Sr (ZJK40 and ZJK41), which were processed using the same conditions as described in Example 3. An alloy with the addition of Zn to the Mg-Y-Zr-Ca system (WZKX42 alloy) was also compared to the other alloy systems. For cytotoxicity tests, samples were sterilized by ultraviolet radiation for about 1 hour. The below results show that the addition of Sr resulted in a significant improvement in

corrosion resistance as demonstrated by immersion corrosion data while there was no deterioration in the mechanical properties or cytocompatibility.

TABLE 2C

Mechanical Properties of the new alloys containing Sr (ZJK), Zn (ZK), and Y—Zn—Ca—Zr (WZKX42) following improved processing.				
Alloy	Young's Modulus (GPa)	Yield Strength (MPa)	Ultimate Tensile Strength (MPa)	Percent Elongation (%)
Commercial AZ31	55	202	268	12
Pure Mg	5	19	66	7
ZK40 as-cast	64	96	176	4
ZK40 T4	68	92	83	2
ZK40 as-extruded (Mg—Zn—Zr)	45	287	327	9
ZJK with 0.1% Sr (Mg—Zn—Zr—Sr)	42	293	317	13
ZJK40				
ZJK with 1% Sr (Mg—Zn—Zr—Sr)	41	293	321	10
ZJK41				
WZKX42 (Mg—Y—Zn—Zr—Ca)	52	334	412	14

[0129] Mechanical properties of the magnesium alloys used for K-wires were determined using tensile testing. High strength materials of titanium and stainless steels are currently used in K-wires to sustain loads placed on the device to maintain fracture union. Thus, magnesium alloys used to replace these inert metals should also have high strength. Table 2C shows the Young's modulus, tensile strength, and elongation of the strontium-containing magnesium alloys compared to commercially available pure Mg and Mg-Al-Zn (designated AZ31B) magnesium alloy (Goodfellow Corporation, USA), as well as the alloys ZK40 and Mg-Y-Zn-Zr-Ca (designated WZKX42), which have an absence of strontium. The inventive alloys belonging to the ZJK series exhibited significantly higher yield and ultimate tensile strength compared to pure Mg and AZ31. It is important to note that earlier alloys were not extruded while the new alloys have been, which partially contributed to the increase in strength, along with the selection of alloying elements, e.g., the presence of strontium. The high strength of WZKX42 was attributed to the addition of Zn which facilitates the formation of precipitates arranged in a long period stacking order (LPSO), an important factor contributing to impeding the movement of dislocations and enhancing the mechanical strength. The increase of strength of the new alloys is significant to support the favorable use of these alloys in claims of applications in various medical devices which particularly require high strength.

[0130] Vickers microhardness was measured by applying a load of 100 g for 10 s to polished Mg samples and measuring the indentation using optical microscopy. Microhardness of the strontium-containing and WZKX42 alloys were higher than both commercially available Mg and AZ31B alloy.

[0131] Cell viability of MC3T3-E1 pre-osteoblast cells cultured in media containing degradation products from 3 days of alloy immersion was determined using the MTT assay. Extract media from alloys was prepared in accordance with ISO Standard 10993:12. High cell viability was observed, with at least 75% cell viability observed for all

alloys at 10% and 25% dilution of extract after culturing cells with the extract for 1 day and 3 days.

[0132] In vitro immersion corrosion measurements were performed in Hanks' Balanced Salt Solution (HBSS) to assess the corrosion rates of strontium-containing biodegradable magnesium alloys. Commercially available AZ31B and pure Mg were used as control groups in this study. Commercial extruded AZ31B exhibited the lowest corrosion rate (~ 0.05 mmpy) and the magnesium alloys exhibited comparable corrosion resistance.

Example 5

[0133] Pure Mg, Al, Zn, Ca and Mn were melted at 720° C. under a protective environment of Ar+0.1% SF₆ and poured into steel molds preheated at 500° C. The compositions were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES) and reported in Table 3C. The as-cast ingots (AZXM-AC) were then T4 heat treated (AZXM-T4) at 385° C. in Ar atmosphere for 10 h to solubilize any intermetallics and alloying elements in the alloy matrix. The T4 heated ingots were extruded (AZXM-EX) at 300° C. with an extrusion speed of 1 mm/s followed by quenching in water. To study the in vitro degradation behavior of AZXM alloy, round shape plates with a diameter

of 10 mm and thickness of 2 mm were immersed in Hanks' solution for 1, 2 and 3 weeks according to the ASTM G31 standard. Corrosion rate was calculated based on weight loss and the corrosion layer was studied under SEM/EDX. Dog-bone shaped samples were machined for tensile testing. Mechanical properties were measured using the Instron 5900 testing system equipped with an extensometer measuring the elongation. For primary cytotoxicity evaluation, MTT test was conducted on BEAS-2B cell line (human bronchial epithelial cells).

TABLE 3C

Chemical composition of AZXM alloy.					
	Al	Zn	Ca	Mn	Mg
Nominal Composition (wt. %)	2%	1%	0.6%	0.2%	Bal.
Chemical Composition (wt. %)	1.92%	1.05%	0.56%	0.02%	Bal.

[0134] The grain size of as cast AZXM alloy was about 300 μ m and did not significantly change after T4 treatment. However, the grains were greatly refined after conducting hot extrusion. In the transverse directions, typical undefined grains characteristic of extrusion are visible due to severe plastic deformation. The grains were elongated along the extrusion direction.

[0135] AZXM as cast exhibited poor corrosion resistance and exhibited the highest corrosion among all of the test groups. T4 treatment however improved the corrosion resistance.

[0136] The corrosion resistance of AZXM alloys was also significantly improved after extrusion and is also not significantly different from the corrosion rate of AZ31 alloy. ICP data of Mg²⁺ show concentration profiles similar to the corrosion rates from different test groups. However, in some groups, the accumulation of Mg²⁺ was reversed after 2 weeks of immersion because of the deposition of the degradation product. Compared to AZ31, even though the corrosion rate of extruded AZXM was slightly higher, the overall corrosion appeared to be more uniform with the formation of Mg (OH)₂ based on an EDAX analysis. Pitting is the primary corrosion mode exhibited on the AZ31 surface due to the microstructure generated by the extrusion process.

[0137] Mechanical properties (Table 4C) were also significantly improved after extrusion due to the grain refinement following extrusion. The increase in Young's modulus and strength will be useful in providing enable better mechanical support for the fabricated stents, and the improved ductility would certainly help in preventing fracture as a result of the expansion of the stent.

TABLE 4C

Mechanical properties of AZXM alloy.				
	Young's Modulus (GPa)	Yield Strength (MPa)	Ultimate Strength (MPa)	Elongation at Fracture (%)
AZXM-AC	23.80 $\pm$ 5.20	90.75 $\pm$ 7.39	129.00 $\pm$ 12.68	3.63 $\pm$ 0.37
AZXM-T4	31.09 $\pm$ 0.19	84.20 $\pm$ 9.28	133.83 $\pm$ 9.75	3.53 $\pm$ 1.03
AZXM-EX	38.19 $\pm$ 4.26	233.53 $\pm$ 4.15	283.08 $\pm$ 1.72	8.56 $\pm$ 2.05

[0138] Viability of BEAS-2B cells was extremely low when cultured in 100% extract both on day 1 and day 3. However, the cell viability significantly increased when the extract was diluted. The dilution ratio and culture time did not significantly affect the cell viability. This result implies that the impact of AZXM stent on tracheal tissue may be limited, since the degradation product will be diluted by the mucus that continuously flows through the airway.

[0139] AZXM alloy exhibits potential for use as degradable intra-luminal stents for tracheal obstructions. Extruded AZXM alloy shows less pitting, higher corrosion resistance and optimized mechanical properties. Future plans for clinical translation would include intra-luminal tracheal stent design and manufacture, as well as in vivo evaluation in rabbit tracheal stenosis model.

Example 6

[0140] A rat femoral fracture model was used to assess the performance and toxicity of modified-ZK40 (M-ZK40) and Mg-Y-Zn based alloys.

[0141] M-ZK40 and Mg-Y-Zn based alloy rods were inserted into the intramedullary cavity to repair a full osteotomy in the midsection of rat femurs. Ti-6Al-4V rods were used as the negative control. After 7 days, X-ray imaging was performed to confirm the location of the rods. Blood was drawn to perform blood cell counts and serum biochemical test prior to implantation and after sacrifice at 14, 56 and 84 days. After sacrifice, femurs were explanted

and μ CT was used to observe the surrounding bone and measure the degradation rates of the Mg alloy rods. Local tissue response and bone healing was assessed by histology with Goldner's Trichrome staining. Potential systemic toxicity in the liver and kidney was evaluated by H&E staining and measurement of alloying elements by ICP-OES.

[0142] X-ray images of the metallic rods used to fix the full osteotomy demonstrated the placement of the rods in the middle of the femoral intramedullary cavity to align the bone fragments and fix the defect. Blood test results exhibited no significant difference between groups or time points. Blood cell counts, liver and kidney functional parameters, phosphorous, chloride, magnesium, and potassium levels at 0, 14, 56 and 84 days were within the normal range. No sign of accumulation of degradation products or toxicity were observed in liver and kidney histology and elemental analysis. Indications of corrosion were observed at high stress regions near the fracture site, suggesting the onset of stress corrosion cracking. μ CT images after 2 weeks exhibited some breakage of rods in both M-ZK40 and Mg-Y-Zn groups. Despite the fragmentation of the Mg alloy rods, new bone formation was observed between the ends of the fragmented femurs.

[0143] In order to demonstrate the degradation of M-ZK40 and Mg-Y-Zn rods under load-bearing conditions, a rat femoral fracture model was employed. Breakage of rods and indications of corrosion were observed. However, the Mg alloy rods did not appear to cause significant toxicity, with no significant difference between groups and time points shown in blood, liver, and kidney analyses.

Example 7

Alloy Fabrication and Characterization

[0144] Alloy WJ11 was generated by Yueyang Yuhua Metallurgical New Materials Co., Ltd. (Yueyang, Hunan, China) utilizing conventional melting and casting procedures followed by extrusion to yield 16 mm diameter rods. An extrusion ratio of 10 was used. The proposed alloying elements composition and actual fabricated composition as determined using inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP duo 6500 Thermo Fisher, Waltham, MA) are shown in Supplementary Table 1.

SUPPLEMENTARY TABLE 1

The chemical composition of the WJ11 alloy.		
Chemical composition (wt. %)	Proposed	Actual (by ICP)
Yttrium (Y)	1.00	0.909 $\pm$ 0.004
Strontium (Sr)	1.00	1.102 $\pm$ 0.005
Zirconium (Zr)	0.50	0.447 $\pm$ 0.001
Magnesium (Mg)	balance	balance

[0145] Test solution for ICP-OES analysis was prepared by dissolving a known weight of WJ11-extruded rod in a known volume of 3% nitric acid. The solution was then diluted with 3% nitric acid in deionized water to analyze various element concentration. Five standards solutions with known different concentrations of Mg, Sr, Y and Zr elements were prepared using certified single element standard solutions suitable for ICP (Sigma-Aldrich, St. Louis, MO). Nitric acid (3%) in deionized water also used a blank standard. Various crystalline phases present in the extruded WJ11

alloy were determined by X-ray diffraction (XRD) in a Philips X'Pert PRO diffractometer using Cu K α radiation with a Si-detector (X'celerator). The X-ray generator was operated at 45 kV and 40 Ma at a 2 θ range of 10-90 $^{\circ}$ employing a step size of 0.033 and 90.17 s time step. Thin cross-sections of the WJ11 extruded rod were mounted in epoxy resin (EpoxiCure, Buehler) and polished with 320, 600, 1200 grit SiC polishing paper and 3 μ m and 1 μ m Al₂O₃ powder, respectively, to obtain a mirror finish surface. In order to observe the grain structure of the Mg-based alloys, the samples were then subjected to chemical etching in a picral acid solution consisting of 5 ml acetic acid, 6 g picric acid, 10 ml water, and 100 ml ethanol followed by using acetone to clean the surface. The polished and etched surface was observed using an optical microscope (Smartzoom 5, Zeiss, Germany) to obtain the optical microstructure images. The microstructure and precipitates of alloys surface were also observed under the scanning electron microscope (Apreo SEM, Thermo Fisher Scientific). The elemental composition and distribution were analyzed by energy dispersive x-ray spectroscopy (Elite T EDS system, EDAX).

Scaffold Design, Fluoride Surface Coatings, and Sterilization

[0146] Circular cranial implants of 8 mm of diameter and 0.8 mm thickness (based on the rat skull thickness) were fabricated from WJ11 extruded rod and PEEK rod (McMaster-Carr, Catalog: 8503K222, LOT #5321573) using computerized numeric control (CNC) automated machine. All the machined PEEK cranial bone grafts (PEEK-CBG) were repeatedly washed and cleaned with water, isopropanol and acetone under ultrasonication. WJ11 implant surfaces were polished with 320, 600 and finally 1200 grit SiC paper and cleaned under ultrasonication using acetone. The polished WJ11 substrates were soaked in 5 M NaOH solution at 60 $^{\circ}$ C. for 2 h, then cleaned thoroughly with deionized water and dried at 60 $^{\circ}$ C. These hydroxide-treated WJ11 substrates were then immersed in HF (48-51 wt. %, Acros Organics) at room temperature for 72 h under constant stirring within a fume hood. The MgF₂-coated WJ11 cranial bone grafts (denoted by FWJ11-CBG) were first washed thoroughly with distilled water and then with acetone, and were finally dried in air at 50 $^{\circ}$ C. for 20 min. The implants were sterilized by gamma radiation (2 $\times$ 10⁶ cGy, 23.5 Gy/min, cesium 137 source, Mark I 68, JL Shepherd and Associates, San Fernando, CA, USA).

Immersion Corrosion Test

[0147] ASTM standard G31-12a was used to evaluate the in-vitro degradation of the polished bare WJ11 and fluoride coated WJ11 bone grafts in Hank's balanced salt solution (HBSS, Sigma-Aldrich and Product Number H1387) at 37 $^{\circ}$ C. The surface area and volume of each of the samples were determined from micro-computed tomography. Each sample was weighed before immersion and immersed in HBSS in a 50 mL conical centrifuge tubes and the ratio of HBSS volume to sample surface area was 0.20 mL/mm². After 1, 3, and 5 weeks of immersion the samples (n=4/time point) were carefully retrieved from the HBSS media, washed in DI water, acetone and dried in air. The corrosion products were removed by immersion in solution of 200 g of chromium trioxide, 10 g of silver nitrate, and 1000 mL of water for 3-5 min. The samples were then washed in DI water and

acetone, dried in air, and weighed. The corrosion rate following ASTM standard G31-12a was calculated based on the mass loss utilizing equation 1 as given below

$$\text{In-vitro corrosion rate (mm/year)} = K \frac{W}{ATD} \quad (1)$$

[0148] Where, the constant K is 8.76×10^4 , W is mass loss (g), A is the surface area of each sample (cm^2), T is the immersion time (hour), and D is the density of each alloy (g/cm^3). The sample surface after removing the corrosion product was observed by SEM.

Surgical Model and Study Design

[0149] The surgical procedure was developed following published literature and all the animal experiments were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC). Before calvarial surgery, male Sprague-Dawley rats weighing 250-300 g were anesthetized by inhalation of isoflurane at a concentration of 4-5% coupled with oxygen for induction of sedation and 1.5-2.5% for maintenance. A subcutaneous injection of buprenorphine (0.05 mg/kg) for postoperative analgesia was given, and the animal was transferred onto a heating pad (maintained at 37°C .) in the operating table. All procedures used sterile instruments, surgical gloves and aseptic procedures. By using the scalpel, ~1.5 cm incision was made down to the periosteum over the scalp from the nasal bone to just caudal to the middle sagittal crest or bregma. The periosteum covering the calvarium down the sagittal midline was sharply divided with the scalpel, and the periosteum was gently pushed laterally while elevating from the underlying skull. A self-retaining retractor or manually retractor was used to spread the soft tissues and expose the underlying bone. A trephine with an 8 mm diameter (ACE Surgical Supply Co., Inc., USA, Product number: 04-9487-01) was used to cut the calvarial bone and make an 8 mm defect. The trephine and calvarium were irrigated with sterile normal saline dropwise throughout the whole drilling procedure. The elevator was used to gently lift the bone from

the dura, first by lifting the edge and then by running the elevator under the surface to free any adherent dura. After thoroughly rinsing with physiological saline to wash out any bone fragments, the sterilized scaffold was placed carefully into the defect and each animal received only one implant. The periosteum over the implant was closed using a running 4-0 Vicryl suture (Ethicon, Product: J422H) and the skin over the periosteum was also closed using 4.0 Vicryl suture. Post-operative pain and distress was assessed through observation for stressed expression and for behavioral abnormalities such as excessive scratching and rubbing of the wound. In addition, the rats were monitored for failure of oral intake, lethargy, and abnormal walking. Post-operative pain was controlled with subcutaneous administration of buprenorphine (0.05 mg/kg every 12 hours) for 72 hours. All animals received postoperative subcutaneous injections (2 mg/kg, twice a day) of Baytril for 5 days prophylaxis for infection. At 4, 8, 12 and 26 weeks after surgery, rats were euthanized by overdose of CO_2 followed by a bilateral thoracotomy. Death was confirmed by a lack of heartbeat and faded eye color. Once heartbeat stoppage was confirmed blood collection was conducted by cardiac puncture and then a rectangular piece of bone tissue along with the implant was carefully harvested using a saw (Sabo Saw, Stryker, USA) for Micro-computed tomography ($\mu\text{-CT}$) imaging and histological analysis. The explants were stored in 70% ethanol at 4°C before decalcification. In addition, liver, kidney, spleen and brain were also collected for histopathological toxicity evaluations.

[0150] Groups of five animals ($n=5$) for both PEEK-CBG and FWJ11-CBG were used for each time point of 2, 8, and 16 weeks, and ten animals ($n=10$) for each scaffold group was used for 26 weeks' time points. The number of animals ($n=5$) used per group per time point were determined following published literature reported on testing of Mg alloys and evaluating their corrosion, biocompatibility, local and systemic toxicities. Other publications reporting on using Mg alloys in rat calvarial regeneration also used a sample size of $n=5$. The sample size of each analysis for each scaffold group per time point is given in Supplementary Table 2.

SUPPLEMENTARY TABLE 2

Summary of number of explants in each group at a certain time point used to analyze new bone formation, scaffold degradation and toxicity.					
Analysis technique	Group	Time point			
		4 weeks	8 weeks	16 weeks	26 weeks
μCT (new bone formation and degradation)	PEEK-CBG	$n = 5$	$n = 5$	$n = 5$	$n = 10$
	FWJ11-CBG	$n = 5$	$n = 4$	$n = 5$	$n = 8$
Blood cell count and serum biochemical tests	PEEK-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
	FWJ11-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Bone histology	PEEK-CBG	$n = 1$	$n = 1$	$n = 1$	$n = 1$
	FWJ11-CBG	$n = 1$	$n = 1$	$n = 1$	$n = 1$
Histopathology of brain	PEEK-CBG	$n = 5$	$n = 5$	$n = 5$	$n = 7$
	FWJ11-CBG	$n = 5$	$n = 5$	$n = 5$	$n = 7$
Histopathology of kidney	PEEK-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
	FWJ11-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Histopathology of Liver	PEEK-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
	FWJ11-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Histopathology of spleen	PEEK-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
	FWJ11-CBG	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Biomechanical Push-out tests	PEEK-CBG	—	—	—	$n = 5$
	FWJ11-CBG	—	—	—	$n = 4$

Micro-Computed Tomography (μ -CT) Imaging, New Bone Volume and in-Vivo Degradation

[0151] Micro-CT was primary used to analyze the volume of the bare and FWJ11-CBG to reflect the in-vitro and in-vivo degradation profile and degradation rates of the corresponding implants. Bare WJ11, FWJ11-CBG, and harvested calvarial samples were placed in a sample holder to prevent movement during scanning and were scanned with continuous rotation at 23 μ m voxel size resolution (Siemens Inveon Multi-Modality microPET/SPECT/CT, Voltage: 80 kv, Current: 500 μ A, Exposure: 1250 ms). The files were converted into the digital imaging and communications in medicine (DICOM) format and analyzed using the Mimics software (Mimics Research 21.0 and 3-matic research, Materialise, Belgium). A threshold was set to isolate the implant from the surrounding tissues and the region growth function was used to identify the remaining structure of the stent which was then reconstructed into 3D images of all the stents. The Dynamic Region Grow tool and a threshold of 350-1400 Hounsfield units (HU) was used to calculate the volume of the bare WJ11 and FWJ11-CBG for in-vitro degradation studies and before calvarial implantation surgeries. New bone volume of calvarial explants implanted with PEEK-CBG harvest at various time points were analyzed using a HU scale in the threshold range of 580-1250 (HU scale range predefine as "Compact Bone (CT, child)" in Mimics). PEEK appears radio transparent to x-ray in this HU range and does not contribute to the new bone volume determination process. The new bone volume formed inside the defect was calculated by measuring the total volume of the explant (V_{ex}) and measuring the volume of the native bone (V_{ntb}) only after removing the new bone formed inside the 8 mm calvarial defect. This can represent by:

$$\text{Volume of new bone } (V_{nb}) = V_{ex} - V_{ntb} \quad (2)$$

[0152] The new bone was removed carefully from each μ CT image layer using the segment-multiple slice edit tool to avoid any removal of the native bone. The defect volume was calculated by measuring the defect area from the μ CT image and multiplying the value with the constant scaffold thickness value of 0.8 mm. The radiopacity or radiodensity of natural bone, newly formed bone, and FWJ11-CBG appear to be similar in μ CT images, therefore, using cylindrical volume of interest technique as reported in the literature cannot be applied. Therefore, a method was developed in which the newly formed bone was removed very carefully from each layer of the each μ CT image layer using the segment-multiple slice edit tool to avoid any removal of the native bone or FWJ11-CBG. In the second stage the FWJ11-CBG was removed from each layer. The volume of new bone (V_{nb}) and volume of remaining un-degraded FWJ11-CBG (V_{FWJ11}) were calculate using equations 3-4.

$$\text{Volume of explant } (V_{ex}) = V_{ntb} + V_{nb} + V_{FWJ11} \quad (3)$$

$$\text{Volume of new bone } (V_{nb}) = V_{ex} - (V_{ntb} + V_{FWJ11}) \quad (4)$$

$$\text{Volume of un-degraded FWJ11-CBG } (V_{FWJ11}) = V_{ex} - (V_{ntb} + V_{nb}) \quad (5)$$

[0153] The volume loss (%) of a specific graft at a particular time point was calculated using equation 6:

$$\text{Volume loss (\%)} \text{ of FWJ11-CBG} = \frac{(V_{FWJ11}^i - V_{FWJ11}^T) \times 100}{V_{FWJ11}^i} \quad (6)$$

[0154] Where V_{FWJ11}^i is the initial volume of the graft and V_{FWJ11}^T is the volume of the graft at a certain post-implant time point. The defect volume was calculated by measuring the defect area from the μ CT image and multiplying the value with the constant scaffold thickness value of 0.8 mm. The corrosion rate was calculated by modifying equation 1 as given below:

$$\text{In-vivo corrosion rate (mm/year)} = K \frac{\Delta V_{FWJ11}}{AT_{imp}} \quad (7)$$

[0155] Where ΔV_{FWJ11} is change in the volume or volume loss (cm^3) of the FWJ11-CBG after a certain implantation time point, K is 8.76×10^{-4} , A is the initial sample area exposed (cm^2) and T_{imp} is the implantation time point in hours (h).

Blood Cell Count and Serum Biochemical Measurements

[0156] Whole blood cell counts on bloods samples collected by cardiac puncture were performed by Marshfield Labs (Cleveland, OH, USA) using a Sysmex XT2000i Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan) with provided reference ranges. Serum samples were obtained by centrifuging collected blood at 2000 r/min for 10 min at 4° C. Serum biochemical tests were conducted by Marshfield Labs using an Olympus AU chemistry analyzer (Olympus Corporation, Tokyo, Japan) with reported reference ranges established by Marshfield Labs. Values of the sham control were taken from our published results.

Histological Sample Preparation and Analysis

[0157] Specimens of brain, spleen, liver and kidney were fixed in 10% neutral buffered formalin (NBF), dehydrated, cleared, infiltrated and embedded in paraffin. Blocks were sectioned at 7 microns, stained with hematoxylin and eosin (H&E), and investigated for cell infiltration, tissue morphology, and pathological changes due to the degradation and clearance of the WJ11 alloy in these critical visceral organs. Local and systemic toxicity analyses on the brain, liver, kidney and spleen were performed by a board-certified veterinary pathologist in a blinded fashion to avoid any study bias.

[0158] Calvarial samples were fixed in 10% NBF and subsequently decalcified using Surgipath Decalcifier-I decalcifying solutions (Leica Biosystems, USA). The paraffin embedded blocks were sectioned at 7 microns with a rotary microtome (Leica RM 2255, Leica Biosystems, Buffalo Grove, IL, USA) and stained using H&E (Leica Biosystems, Buffalo Grove, IL, USA), Picrosirius red (Polysciences, Inc. USA, Cat. No. 24901-250), and Goldner's Trichrome (Merck KGaA, Germany, Cat. No. 1.00485.0001) stains to observe bone morphology and osteoblast activity at the defect site. Protocols provided by the manufacturers were used to stain the slides. Histology sections of various tissues were imaged by an inverted microscope with the fluorescence illuminator (CKX41, Olympus, Olympus America Inc.). To distinguish the maturity of the collagen in the defect, picrosirius red stained images were captured by using polarized microscopy (Olympus Provis 1 Fluorescence microscope). No quantitative analysis of the histological data was performed.

Tensile, Compressive and Mechanical Push-Out Testing

[0159] Tensile bars were machined along the axis direction of the extruded rods by CNS machine. The size of the tensile bars was determined as per ASTM E8/E8M-15a standard with 25.0 ± 0.1 mm gauge length and 6.0 ± 0.2 mm $\times$ 6.0 ± 0.2 mm gauge area. For each sample, the stress—strain curve was generated by Instron model 3369 servoelectric universal testing machine (Instron, Norwood, MA) equipped with an extensometer (Instron 2630 series, Norwood, MA) measuring the elongation. A 10 kN load cell and a crosshead displacement rate of 0.25 mm/min was used for all the tests. Seven (n=7) typical stress-strain curves obtained from each group were used to calculate the yield strength (YS), ultimate strength (UTS) and elongation at fracture (EL) using the Bluehill 3 Testing Software for Mechanical Testing Systems (Instron, Norwood, MA). Compressive tests were performed on cylindrical specimens of 20 ± 0.2 mm of height and 10 ± 0.1 mm of diameter following ASTM E9-19 test method. An Instron model 3369 servoelectric universal testing machine using a 50 kN load cell and a crosshead displacement rate of 1.27 mm/min was used for compression testing. All the specimen (n=10) failed by fracture and the compressive strength was calculated by measuring the maximum stress before fracture.

[0160] Explanted 26 weeks calvarial samples were stored frozen in saline-soaked gauze at -20° C. and push-out testing was performed after thawing to 25° C. Push-out tests were performed on Instron 5500 unit (Instron, Norwood, MA) using a custom-made push-out system similar to puncture test kit for packaging material provided by Intron (Catalog no. S1-11855). The push-out rod with a diameter of 6.0 mm was used. The diameters of the upper and lower platens holes were 7 mm and 10 mm, respectively. The diameter of the lower platen was chosen to allow 1.0 mm clearance between the construct and the supporting plate to minimize the effect of the platen-based support system on the interfacial stress distribution. Explants were oriented with the construct centered over the lower platen hole and push-out rod loaded with SOON load cell applied a vertical force on the constructs at a constant rate of 0.5 mm/min. Testing was stopped after a peak force was reached. The interfacial shear strength was calculated using the following formula:

$$S = \frac{F}{\pi \times d \times t} \quad (8)$$

[0161] Where S is the interface shear strength (MPa), F is the peak push-out force (N), d is the diameter of the scaffold (i.e., 8.0 mm) and t is the average bone/scaffold thickness (i.e., 0.8 mm).

Statistical Analyses

[0162] All the plots and statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc.). Continuous variable data are represented as means $\pm$ standard deviation. Following the recently published recommenda-

tion the a priori (α) defined significance level for all statistical tests was 0.05. A one-way ANOVA followed by Bonferroni Procedure post hoc tests were conducted for in-vitro corrosion, formation of new bone volume, and in-vivo FWJ11 volume loss to determine the differences between the different groups of samples. The peak stress and interfacial shear strength obtained from the mechanical push-out test data was compared using unpaired t-test (two tailed).

Results

Composition, Phase, Microstructure and Mechanical Properties of the WJ11 Alloy

[0163] The extruded WJ11 was characterized for the properties relevant for use as calvarial implant. The elemental composition of the extruded WJ11 alloy, as determined by the ICP and shown in Supplementary Table 1, are very close to the nominal composition. Other impurities failed to be detected, such as iron or manganese presence in this alloy using ICP and therefore minimizing any rapid degradation caused by microgalvanic corrosion. The crystalline phase of the extruded WJ11 alloy was determined using XRD. The XRD pattern of WJ11 is similar to that of pure hexagonal (α -phase) crystalline Mg and exhibits absence of any other intermetallic phases. The SEM image of the etched surface perpendicular to the extrusion direction showed the presence of grains of α -phase of sizes mostly below 5 μ m and precipitates around the grain boundary. SEM images also showed presence of small 2-5 μ m precipitates around the grain. EDX analyses on these precipitates show that these precipitated particles contain more strontium, than the matrix. This may be due to the limited solubility of Sr in magnesium. The tensile and compressive strength of the as extruded WJ11 bars were measured and values of various mechanical properties are shown in Table 5. The tensile yield strength, ultimate tensile strength and compressive strength of the alloy is very similar to various biodegradable strontium and rare earth containing Mg alloys reported in the literature for various orthopedic applications.

TABLE 5

Tensile and compressive mechanical properties of WJ11	
Elastic modulus (GPa)	46.7 ± 10.95
Yield strength (MPa)	175.6 ± 3.11
Ultimate tensile strength (MPa)	181.8 ± 3.92
Strain at failure (%)	2 ± 0.99
Compressive strength (MPa)	301.96 ± 13.83

In-Vitro Corrosion of Fluoride Coated WJ11 Cranial Bone Grafts (FWJ11-CBG)

[0164] Fluoride coated WE 1 cranial bone grafts (FWJ11-CBG) and PEEK cranial bone grafts (FWJ11-CBG) were fabricated from as extruded WJ11 alloy rods and PEEK rod. The surface morphology of the as polished bare WJ11 cranial bone graft and FWJ11-CBG was observed by SEM. The presence of fluoride coating was confirmed by SEM-EDX. Fluoride treatment leads to formation of a thin fluoride coating along with several fine pores of <5 μ m of diameter

on the surface of the FWJ11-CBG. This may be due the dissolution of the fine intermetallic precipitates or oxides from the surface of the bare substrates. In-vitro corrosion rates were calculated using equation-1 of the bare and fluoride coated bone grafts under HBSS at 37° C. for 7, 21 and 35 days of immersion. The corrosion rate for both groups decreases with time and the corrosion rate of bare alloys is almost six to nine times higher than the fluoride coated bone grafts. SEM images of the corroded surface after 35 days of immersion of the bare and fluoride coated grafts showed that the number of corrosion pits and depth of the corrosion pits on bare WJ11 surfaces is much higher as compared to FWJ11-CBG. Moreover, it appeared that corrosion occurs more frequently near the edges than at the bulk of the grafts.

Animal Surgery and Harvesting of Explants

[0165] The in-vivo osteogenic properties of the FWJ11-CBG and PEEK-CBG were investigated in rat 8 mm of diameter critical-sized calvarial defect for 4, 8, 16 and 26 weeks. Out of 52 animals, 2 animals implanted with PEEK-CBG died within 24 hours of surgery due to surgery related complications. All other animals survived the entire duration of the study and no symptoms related to inflammations, or infections were observed. The FWJ11-CBG sample group also had no specific symptoms such as swelling or dehiscence due to hydrogen gas formation. Both of the groups did not show any sign of visual changes in the overlying tissue, underlying brain tissue, and other surrounding tissues and skin.

Rat Calvarial Defect Regeneration Using μ CT

[0166] The role of implanted samples on bone formation at the critical sized defect was evaluated using μ CT as a nondestructive method. The 3D reconstructed μ CT of the explants collected at 4, 8, 16 and 26 weeks for the PEEK-CBG group was obtained. The formation of new bone was separated from original native bone from original 3D reconstructed μ CT data in Mimics software. The μ CT of the FWJ11-CBG explants collected at 4, 8, 16 and 26 weeks was obtained. In case of explants from FWJ11-CBG group, the formation of new bone was separated from original native bone and the remaining FWJ11 graft. The μ CT images indicate formation of bone around the outer volume of the implants as throughout the channels of the bone grafts. At time points of 4 and 8 weeks, μ CT images revealed very small bone growth around the outer edges of most of the PEEK and FWJ11 grafts. At longer time points (16 and 26 weeks) the integration of the grafts with surrounding tissue improved, however, complete osseointegration was not observed for any of the grafts from either of the groups. 3D reconstructed μ CT images of the explants also indicated that at 4 and 8 weeks the newly bone formed at the channels and on the surface of the FWJ11 and PEEK grafts were not connected to the surrounding native bone. However, more continuous new bone formation was observed from surrounding native bone to the surface and channels of the explant grafts at 16 and 26 weeks. The 3D reconstructed

μ CT images also indicated that the formation of bone started mostly on the surface in contact with the dura mater rather than the surface in contact with the periosteum and skin. The volume of new bone formed inside the critical sized calvarial defects were calculated from 3D reconstructed μ CT data using equation-2 in case of PEEK-CBG and using equation-4 for FWJ11-CBG implants. The amount of new bone formed and the normalized new bone volume with respect to the total defect volume for both the implant groups was observed. The volume of new bone formed increased with increase in implantation time for both PEEK and FWJ11 calvarial grafts. The new bone formation at a certain time point for a specific group varied greatly from animal to animal and there are no statistically significant differences in the new bone volume within any of the time points. The percentage of normalized new bone formation increased from 6.7% ($\pm 4.1\%$) at 4 weeks to 16.8% ($\pm 4.9\%$) at 26 weeks for FWJ11-CBG group, whereas on the other hand, it increased from 2.5% ($\pm 1.6\%$) at 4 weeks to 13.8% ($\pm 2.4\%$) at 26 weeks for PEEK-CBG group. The normalized new bone volumes for PEEK-CBG at 4, 8, and 16 weeks it should be noted, were significantly lower than the normalized bone volumes for PEEK-CBG and FWJ11-CBG 26 weeks explants. The normalized new bone volumes for FWJ11-CBG at 4, and 8 weeks were however, significantly lower than the normalized bone volumes for FWJ11-CBG at 26 weeks. It should be noted that the number of samples used for statistical calculation for each group at each time point are different (see Supplementary Table 2) due to the displacement of some of the FWJ11 grafts from the defect site.

Evaluation of New Bone Formation via Histological Staining

[0167] The formation of new bone in the channels of the FWJ11-CBG and PEEK-CBG was evaluated using H&E staining on the decalcified section of the explants at various time points. Bone generally presents as a compact structure in a dark red to pink color, connective tissue as light pink porous network and the graft, which was removed during decalcification as white. Masson-Goldner trichrome staining was used to identify tissue by different coloring as well as by morphological identification. New bone formation in the area of the defect was present in all sections examined aside from PEEK-CBG 4 weeks' sample. New bone can be characterized by dense eosinophilic (HE) or green (trichrome) matrix surrounded by osteoblasts representing primary ossification centers. Osteoid was formed in areas of well-vascularized connective tissue without a cartilage intermediate indicating that new bone was formed through intramembranous ossification. Inflammation was not a prominent feature of these histology sections as only scattered inflammatory cells were seen within the connective tissue. Rarely, multinucleated giant cells could be found at the periphery of implant materials; however, more often, implants were surrounded by dense connective tissue. Each H&E and Masson-Goldner trichrome stained calvarial section was examined by the veterinary histopathologist and major findings are listed in Supplementary Table 3.

SUPPLEMENTARY TABLE 3

Summary of histopathological examination of the H&E and Masson-Goldner trichrome stained calvarial section to evaluate new bone formation and implants related inflammation. Capillary score - numbers of capillary profiles in the collagen adjacent to the implant. New bone area - measurement performed using Olympus cell Sens standard imaging software and Olympus DP27 camera		
Bone graft type	Time point	Semi-quantitative histopathological evaluations
FWJ11-CBG	4 weeks	Capillary score - 71 Inflammation - Mild: macrophage, lymphocyte, plasma cell New Bone area - 0.004035823 cm ²
PEEK-CBG	4 weeks	Capillary score - 122 Inflammation - multinucleated giant cells around implant but none in connective tissue New Bone area - none in this section
FWJ11-CBG	8 weeks	Capillary score - 69 Inflammation - Mild: mast cell, macrophage, lymphocyte, plasma cell New Bone area - 0.000428952 cm ²
PEEK-CBG	8 weeks	Capillary score - 80 Inflammation - Minimal: mast cell, macrophage (hemosiderin laden) New Bone area - 0.004011751 cm ²
FWJ11-CBG	16 weeks	Capillary score - 70 Inflammation - Mild: mast cell, macrophage, lymphocyte, plasma cell New Bone area - 0.016688732 cm ²
PEEK-CBG	16 weeks	Capillary score - 94 Inflammation - Mild: macrophage (often hemosiderin laden), lymphocyte, plasma cell New Bone area - 0.005528116 cm ²
FWJ11-CBG	26 weeks	Capillary score - 58 Inflammation - No significant inflammation in defect/implant area New Bone area - 0.011341126 cm ² Other - Osteoblasts lining approximately 1/4 of new bone
PEEK-CBG	26 weeks	Capillary score - 53 Inflammation - Minimal - mast cells, macrophages New Bone area - 0.01282673 cm ²

[0168] Picrosirius red stained sections were used to examine the orientation of collagen by utilizing its natural birefringence under polarized light. Polarized light microscopy allows one, which is enhanced by picrosirius red. In picrosirius red stained sections, well-ordered collagen (type 1) in the native bone and in regions of the newly formed bone appeared green while disordered type 1 collagen appeared yellow/red. The results clearly indicates that the newly formed bone at 4 weeks is mainly unorganized type 1 collagen whereas the amount of unorganized collagen decreases with time and the collagen fibers are very well organized especially at 16 and 26 weeks for both the FWJ11 and PEEK grafts.

In-Vivo Corrosion of FWJ11 Calvarial Bone Grafts

[0169] The 3D reconstructed μ CT images of the FWJ11-CBG explants at 4 and 8 weeks appears intact, however, clear degradation of the FWJ11 graft at 16 and 26 weeks was

easily observed. Progressive degradation of the FWJ11 graft was analyzed from μ CT data. The μ CT images indicate that most of the grafts start to degrade mainly from the rim, the thinnest section of the grafts, and get detached from the main body. The most degraded FWJ11-CBG has lost almost half of its volume after 26 weeks. The volume loss (%) of FWJ11-CBG were calculated using equations 3-6 and the corrosion rate (millimeter per year or mmpy) was calculated using equation 7. The volume loss (%) due to degradation of the FWJ11-CBG increased with increase in implantation time from 8.86 (± 2.3) at 4 weeks to 29.89 (± 10.47) at 16 weeks and decreased slightly to 24.23 (± 11.99) at 26 weeks. The variation in graft degradation among the grafts at 8, 16 and 26 weeks is very large which results in significantly large standard deviations. There is no statistically significant difference in corrosion rates amongst the grafts implanted for 4, 8 and 16 weeks and the corrosion rate significantly decreased at 26 weeks compared to 4 weeks.

TABLE 6

Average blood cell counts of unoperated animals (sham control) and animals implanted with FWJ11-CBG and PEEK-CBG at 4, 8, 16 and 26 weeks after implantation.					
Name	Implantation time	Red Blood Cell Count	Hemoglobin	Platelet Count	White Blood Cell Count
Units		10 ⁶ /μL	g/dL	10 ³ /μL	10 ³ /μL
Ref. ranges		(7.00-9.00)	(13.7-16.8)	(680-1280)	(1.1-7.5)
Sham control		7.7 ± 0.4	14.9 ± 0.5	877 ± 55	12.1 ± 3
FWJ11	4 weeks	8.5 ± 0.3	15.7 ± 0.4	735.3 ± 145	13.5 ± 5
PEEK	4 weeks	8.6 ± 0.4	15.3 ± 0.8	747.3 ± 72	11.4 ± 4
FWJ11	8 weeks	8.5 ± 0.2	15.0 ± 0.3	652.7 ± 156	8.43 ± 2
PEEK	8 weeks	7.8 ± 0.5	13.8 ± 0.4	840.3 ± 51	12.7 ± 1
FWJ11	16 weeks	8.8 ± 0.1	14.4 ± 0.5	814.3 ± 48	16.3 ± 2
PEEK	16 weeks	9.1 ± 0.2	15.2 ± 0.4	1062.3 ± 50	14.4 ± 1
FWJ11	26 weeks	9.0 ± 0.3	15.0 ± 0.3	641.1 ± 131	15.1 ± 2

Systemic Toxicity to FWJ11-CBG Implants

[0170] Total blood cell counts for all the groups at each time points are listed in Table 6, except for PEEK-CBG at 26 weeks. The bloods samples for this timepoint went bad during transportation from animal facility to the analyzing vendor. The blood cell count results generally did not reveal any disturbance in the blood count values, with all param-

eters remaining within the reference ranges or near the pre-operation (Sham control) levels. White blood cell counts for all the groups at all the times point including the sham control were always higher than the reference range. However, all the operated white blood cell count was within the range of the sham control and therefore not due to any underlying infections.

TABLE 7

Average values of serum metabolic parameters of unoperated animals (sham control) and animals implanted with FWJ11-CBG and PEEK-CBG at 4, 8, 16 and 26 weeks after implantation.						
Name	Implantation time	Glucose	ALT(GPT)	ALP	Total Bilirubin	Total Protein
Units		mg/dL	U/L	U/L	mg/dL	g/dL
Ref. ranges		(70-308)	(59-166)	(232-632)	(0.0-0.1)	(5.8-7.1)
Sham control		168 ± 38	54 ± 6	282 ± 49	0.2	7.2 ± 0.2
FWJ11	4 weeks	210 ± 78	48 ± 10	296 ± 76	0.2 ± 0.0	7.0 ± 0.1
PEEK	4 weeks	297 ± 21	86 ± 39	379 ± 80	0.2 ± 0.0	7.4 ± 0.5
FWJ11	8 weeks	309 ± 128	57 ± 5	243 ± 9	0.2 ± 0.0	7.0 ± 0.1
PEEK	8 weeks	295 ± 98	94 ± 31	237 ± 60	0.2 ± 0.0	7.2 ± 0.1
FWJ11	16 weeks	339 ± 69	335 ± 406	155 ± 51	2.6 ± 3.0	7.2 ± 0.2
PEEK	16 weeks	396 ± 62	211 ± 234	194 ± 44	0.2 ± 0.0	7.9 ± 0.1
FWJ11	26 weeks	319 ± 98	79 ± 32	228 ± 70	0.2 ± 0.0	7.7 ± 0.1
PEEK	26 weeks	340 ± 41	67 ± 26	152 ± 42	0.2 ± 0.0	7.5 ± 0.3

Name	Albumin	Urea N	Creatinine	Globulin	A/G Ratio
Units	g/dL	mg/dL	mg/dL	g/dL	
Ref. ranges	(3.2-3.7)	(13-19)	(0.3-0.5)	(2.6-3.5)	
Sham control	3.5 ± 0.1	20.2 ± 1.3	0.35 ± 0.1	3.71 ± 0.1	0.9 ± 0.05
FWJ11	3.7 ± 0.1	21.3 ± 0.5	0.3 ± 0.08	3.4 ± 0.1	1 ± 0.0
PEEK	3.9 ± 0.2	23.3 ± 2	0.4 ± 0.08	3.4 ± 0.3	1 ± 0.1
FWJ11	3.4 ± 0.1	23.3 ± 1	0.5 ± 0.1	3.6 ± 0.2	0.9 ± 0.0
PEEK	3.5 ± 0.1	16.6 ± 9	0.4 ± 0.04	3.7 ± 0.1	1 ± 0.00
FWJ11	3.6 ± 0.1	20.0 ± 0.8	0.3 ± 0.04	3.6 ± 0.2	1 ± 0.0
PEEK	3.8 ± 0.1	22 ± 0.0	0.3 ± 0.04	4.0 ± 0.0	1 ± 0.0
FWJ11	3.8 ± 0.1	20 ± 1	0.4 ± 0.04	3.9 ± 0.2	0.9 ± 0.04
PEEK	3.7 ± 0.1	19 ± 0.5	0.4 ± 0.05	3.9 ± 0.2	0.9 ± 0.04

[0171] Serum biochemical parameter for kidney function (creatinine and urea levels) and liver functions (albumin (A), alkaline phosphatase, bilirubin, glucose, and globulin (G)) are shown in Table 7 above. All the parameters measured remained within the reference ranges or near the control levels, demonstrating little effect of the implanted alloy materials on the kidney and liver function as well as the metabolism. Electrolyte parameters such as calcium, sodium, chloride, phosphorus, and Mg were also measured from the serum samples and their values are shown in Table 8 below. All the ion concentrations remain in the prescribed allowable ranges including Mg ion indicating no accumulation of the degrading Mg from the implants in the blood.

tions were recorded. Conventional light microscopy images FWJ11-CBG and PEEK-CBG revealed that the cellular structure of the liver, kidney, spleen and brain, except in few animals, did not undergo any notable morphological changes or infiltration by inflammatory cells due. No signs of any obvious abnormalities related to WJ11 alloy degradation were observed in any of the organ sections including brain tissues. The detailed observations for each tissue sample at four different time points are listed in Supplementary Tables 4-7. The abnormalities observed in liver, kidney and brain tissues of a few samples were present for both the implant groups, therefore, the abnormalities were highly unlikely due to the alloying elements released from the WJ11 alloy.

TABLE 8

Average values of electrolyte parameters of unoperated animals (sham control) and animals implanted with FWJ11-CBG and PEEK-CBG at 4, 8, 16 and 26 weeks after implantation.						
Name	Implantation time	Calcium	Sodium	Chloride	Phosphorous	Mg
Units		mg/dL	mmol/L	mmol/L	mg/dL	mg/dL
Ref. ranges		(9.5-13.9)	(146-151)	(98-104)	(5.6-16.8)	(3.8-5.5)
Sham control		12.2 ± 0.3	146 ± 1	98 ± 1	12.1 ± 1.2	4 ± 0.2
FWJ11	4 weeks	12.8 ± 0.2	144 ± 1	98 ± 1	13.3 ± 0.8	4.4 ± 0.3
PEEK	4 weeks	12.8 ± 0.4	139 ± 2	96 ± 2	15.5 ± 1.0	4.8 ± 0.4
FWJ11	8 weeks	12.8 ± 0.2	143 ± 2	97 ± 1	16.9 ± 3.2	4.4 ± 0.5
PEEK	8 weeks	12.7 ± 0.4	141 ± 1	97 ± 1	12.1 ± 1.2	4.1 ± 0.3
FWJ11	16 weeks	12.9 ± 0.6	142 ± 1	97 ± 1	12.6 ± 0.4	4.5 ± 0.3
PEEK	16 weeks	13.1 ± 0.4	143 ± 1	96 ± 1	12.3 ± 0.5	4.9 ± 0.1
FWJ11	26 weeks	13.2 ± 0.4	143 ± 2	97 ± 2	12.2 ± 0.9	4.3 ± 0.3
PEEK	26 weeks	12.1 ± 0.6	147 ± 1	99 ± 2	11.3 ± 0.2	4.1 ± 0.2

Histological Toxicity Examination of Liver, Kidney, Spleen and Brain to FWJ11-CBG Implants

[0172] The blinded H&E stained tissue samples were examined by a veterinary histopathologist and the observa-

SUPPLEMENTARY TABLE 4

Summary of histological examination to evaluate implants related local and systemic toxicities on liver, kidney, spleen, and brain for FWJ11-CBG and PEEK-CBG at 4 weeks.				
Rat Number (group)	Liver	Kidney	Spleen	Brain
R-55 (FWJ1-CBG)	mild centrilobular hepatocellular fatty change	No significant lesions (NSL)	NSL	NSL
R-79 (FWJ11-CBG)	NSL	NSL	NSL	NSL
R-81 (FWJ11-CBG)	Focal moderate hepatic fatty change	NSL	NSL	NSL
R-53 (FWJ11-CBG)	Not analyzed (NA)	NA	NA	NSL
R-54 (FWJ11-CBG)	NA	NA	NA	NSL
R-57 (PEEK-CBG)	mild centrilobular hepatocellular fatty change	1) Multiple mild lymphocytic interstitial infiltrates (medulla). 2) Focal tubular proteinosis (cortex).	NSL	NSL
R-86 (PEEK-CBG)	NSL	Mild multifocal lymphoplasmacytic tubulointerstitial nephritis	NSL	NSL
R-84 (PEEK-CBG)	NSL	NSL	NSL	NSL
R-85 (PEEK-CBG)	NA	NA	NA	NSL
R-56 (PEEK-CBG)	NA	NA	NA	NSL

SUPPLEMENTARY TABLE 5

Summary of histological examination to evaluate implants related local and systemic toxicities on liver, kidney, spleen, and brain for FWJ11-CBG and PEEK-CBG at 8 weeks.				
Rat Number (group)	Liver	Kidney	Spleen	Brain
R-47 (FWJ11-CBG)	Liver - 1) Mild multifocal lymphocytic portal hepatitis. 2) Mild to moderate diffuse hepatocellular fatty change (fat vacuoles in hepatocytes)	Kidney - Focal chronic lymphocytic tubulointerstitial nephritis with tubular loss and mild fibrosis adjacent to an arcuate vein (suspect that this is related to loss of blood flow)	No significant lesions (NSL)	NSL
R-48 (FWJ11-CBG)	NSL	NSL	NSL	Focal perivascular mineral deposition with vacuole (likely in the thalamus)
R-49 (FWJ11-CBG)	NSL	NSL	NSL	NSL
R-13 (FWJ11-CBG)	Not analyzed (NA)	NA	NA	NSL
R-14 (FWJ11-CBG)	NA	NA	NA	NSL
R-50 (PEEK-CBG)	NSL	NSL	NSL	NSL
R-52 (PEEK-CBG)	NSL	NSL	NSL	Focal basophilic foci (mineral vs. fungus - special stain required)
R-51 (PEEK-CBG)	Mild diffuse hepatocellular fatty change (fat vacuoles in hepatocytes)	NSL	NSL	NSL
R-5 (PEEK-CBG)	NA	NA	NA	NSL
R-6 (PEEK-CBG)	NA	NA	NA	NSL

SUPPLEMENTARY TABLE 6

Summary of histological examination to evaluate implants related local and systemic toxicities on liver, kidney, spleen, and brain for FWJ11-CBG and PEEK-CBG at 16 weeks.				
Rat Number (group)	Liver	Kidney	Spleen	Brain
R-27 (FWJ11-CBG)	Minimal to mild centrilobular hepatocellular fatty change	Multifocal mild to moderate chronic lymphoplasmacytic tubulointerstitial and perivascular nephritis	No significant lesions (NSL)	NSL
R-29 (FWJ11-CBG)	NSL	NSL	NSL	NSL
R-28 (FWJ11-CBG)	NSL	NSL	NSL	NSL
R-30 (FWJ11-CBG)	Not analyzed (NA)	NA	NA	NSL
R-31 (FWJ11-CBG)	NA	NA	NA	NSL
R-43 (PEEK-CBG)	Mild multifocal lymphocytic portal hepatitis	NSL	NSL	NSL
R-44 (PEEK-CBG)	NSL	NSL	NSL	NSL
R-45 (PEEK-CBG)	NSL	NSL	NSL	NSL
R-46 (PEEK-CBG)	NA	NA	NA	NSL
R-88 (PEEK-CBG)	NA	NA	NA	NSL

SUPPLEMENTARY TABLE 7

Summary of histological examination to evaluate implants related local and systemic toxicities on liver, kidney, spleen, and brain for FWJ11-CBG and PEEK-CBG at 26 weeks.				
Rat Number (group)	Liver	Kidney	Spleen	Brain
R-17 (FWJ11-CBG)	No significant lesions (NSL)	Mild multifocal tubular degeneration and interstitial lymphocyte infiltrate	NSL	Mild focal white matter vacuolation and adjacent pyknotic cell debris
R-19 (FWJ11-CBG)	NSL	Focal chronic lymphocytic nephritis adjacent to arcuate vessels	NSL	NSL
R-18 (FWJ11-CBG)	NSL	Multiple mineral foci in the renal pelvis	NSL	NSL
R-20 (FWJ11-CBG)	Not analyzed (NA)	NA	NA	NSL
R-22 (FWJ11-CBG)	NA	NA	NA	NSL
R-24 (FWJ11-CBG)	NA	NA	NA	NSL
R-25 (FWJ11-CBG)	NA	NA	NA	NSL
R-37 (PEEK-CBG)	Minimal to mild centrilobular hepatocellular fatty change	NSL	NSL	NSL
R-33 (PEEK-CBG)	NSL	NSL	NSL	NSL
R-38 (PEEK-CBG)	Focal lymphoplasmacytic infiltrate	NSL	NSL	NSL
R-34 (PEEK-CBG)	NA	NA	NA	NSL
R-25 (PEEK-CBG)	NA	NA	NA	NSL
R-36 (PEEK-CBG)	NA	NA	NA	NSL
R-42 (PEEK-CBG)	NA	NA	NA	NSL

Mechanical Push-Out Testing

[0173] Push-out testing results in the form of peak stress and peak interfacial shear strength were obtained. No significant differences in push-out strength were found for the degradable FWJ11-CBG compared to PEEK-CBG. Note that n=4 was used for PEEK-CBG 26 weeks sample due to the shift of the graft from the defect. From the results it appears that the overall effect of degradation of the FWJ11 grafts on biomechanical performance of the explant was insignificant.

Discussion

[0174] Development of biodegradable scaffolds with suitable bone regenerative efficacy and appropriate mechanical properties to reconstruct critical sized calvarial defect defects without the addition of any supraphysiological concentration of exogenous growth factors to promote healing and mineralized tissue formation remains a major challenge. The clinically available calvarial grafts to date with suitable mechanical properties are mostly non-resorbable or partially resorbable and often show various post-surgical complications that tend to have deleterious implications on the surrounding tissue as well as in the implanted host body primarily related to accumulation of particulate debris or delayed type hypersensitivity (DTH). In this context, magnesium based alloys can offer several beneficial advantages over traditional nondegradable metal and polymer based implants due to suitable biocompatibility, biodegradability and biomechanical properties of magnesium and magnesium

alloys. The alloy used in this study contains alloying elements, Sr, Y and Zr (Supplementary Table 1), which are reported to be safe for human use although the long term toxicity data on Y and Zr remain unclear. The positive role of Sr in bone formation by simultaneously promoting osteoblast function and inhibiting osteoclast activity is well established. In fact, Y and Zr containing Mg alloys are already approved for use in human as orthopedic compression screws and vascular stent. Although the exact composition of the alloys used in these CE approved devices are not known, however, it is reported that the amount of Y and Zr can be as high as 5% and 2.5%, respectively. Therefore, the degradation of the WJ11 alloy implant is not expected to elicit any local or systemic toxicological effects due to alloying elements.

[0175] The local toxicity of Mg alloys on the nearby tissue also hinges on the corrosion rate of the alloys. A rapid degradation of Mg alloy-based grafts leads to formation of hydrogen gas pockets and alkaline environment around the implant. Rapid release of hydrogen gas and metallic ions into the local physiological environment can lead to suboptimal bone healing by hindering collagen production and osteoid formation. An increase of local pH due to the release of large amounts of Mg ions has shown to promote the accumulation of calcium phosphate and new bone formation around the implants. However, rapid accumulation of hydrogen bubbles can impede proper connectivity of the osteocytes with the implant surface and hence results in poor bone regeneration. A recent report on a fast-degrading Mg₅Ca alloy implanted in the femoral condyle of rats has also

shown insufficient healing of the bone defect site and possible formation of diseased fibrotic tissue at the implant site. The hydrogen gas bubble generally forms at the early stage (7-30 days) of implantation and gradually disappeared with a moderate inflammatory response and may not affect the overall bone healing. Importance of controlling the degradation of Mg based biomaterials for clinical translation and thereby reducing hydrogen gas pockets formation and rapid release of Mg ions to the surrounding tissue have been recommended for clinical success. In order to circumvent these issues that can arise due to rapid degradation of WJ11, a magnesium fluoride (MgF_2) coating layer was formed on the WJ11 alloy surface. The fluoride coated WJ11 bone graft (FWJ11-CBG) showed a five-to-nine-fold decrease in corrosion rate compared to the bare alloy. Barrier type MgF_2 coating with excellent adhesion strength is known to offer considerable protection against initial rapid corrosion of bare Mg alloys and improves the biocompatibility of the implants. The MgF_2 coated bone implants have been studied by various groups for craniofacial and orthopedic devices showing enhanced biocompatibility and osteogenic activity. It is therefore expected that FWJ11-CBG scaffolds will show slow in-vivo degradation with minimum local and systemic toxicity and help to promote new bone formation in the critical sized calvarial bone defect.

[0176] To evaluate the toxicity and bone regeneration capability of the FWJ11-CBG graft, polyetheretherketone (PEEK), a commonly used graft for craniofacial applications, was used as a control. The polymer, PEEK was chosen because it has mechanical properties similar to bone, as well as excellent thermal and chemical stability, and furthermore, is a nontoxic biomaterial. Therefore, comparison of the bone regeneration capability, local and systemic toxicities of FWJ11-CBG with respect to PEEK-CBG can give a clear picture about the clinical relevancy of Mg alloys-based bone grafts in craniomaxillofacial applications.

[0177] None of the animals implanted with the FWJ11 graft showed formation of hydrogen gas pocket related swelling or dehiscence during the entire study period and the harvested explant showed no sign of changes of the overlying tissues. These results indicate that the fluoride coated FWJ11 graft in-vivo corrosion rate was low enough such that it did not lead to accumulation of excess hydrogen gas. The volume of hydrogen gas formed per day was calculated based on the volume loss of the grafts and was found to be $129 \pm 32 \text{ mm}^3$, $92 \pm 48 \text{ mm}^3$, $112 \pm 38 \text{ mm}^3$, and $54 \pm 28 \text{ mm}^3$ for 4, 8, 16 and 26 weeks of implantation times, respectively. It has been proposed that approximately 130 mm^3 of hydrogen per day can be eliminated by the surrounding tissue for typical implant geometries in well-vascularized locations. Moreover, the short saturation time and high saturation concentration of hydrogen in brain may also favor the rapid clearance of hydrogen from the calvarial site. Few other works on Mg based calvarial implants reported the accumulation of hydrogen at the defect site, however, any comparison was not possible due to the lack of quantitative data on specific alloy degradation or per day of hydrogen evaluation.

[0178] The histopathological examinations of the H&E and Masson-Goldner trichrome stained calvarial sections (Supplementary Table 3) and surround brain tissue (Supplementary Tables 4-7) do not show any hydrogen gas, alkaline pH or alloying ions specific inflammatory reactions. Both the FWJ11-CBG and PEEK-CBG show a predominant

presence of macrophage, lymphocyte, plasma and mast cells. Few multinucleated giant cells were observed around the PEEK-CBG at 4 weeks only. The inflammatory responses observed in this study are as expected and have been reported by others after implanting Mg alloys. Regardless of graft type or time point no polymorphonuclear cells, necrosis, infection, fibrinous exudate, fatty infiltrates, foreign debris, or autolysis were observed (Supplementary Table 3). Formation of fibrous connective tissue and neo-vascularization was observed for all the implants at all the time points. The majority of the newly formed bone was often found to be in direct contact with the implant surface. Histopathology analyses of all the harvested parts of the brain samples which were in direct contact with the calvarial grafts also showed no signs of any lesion or toxicity (Supplementary Table 3) at any time point. All these results clearly suggest that the degradation products and corrosion residues of FWJ11-CBG did not cause any significant and undesired inflammatory reactions in the surrounding tissue including the brain. Although few researchers have tested Mg alloys and related composite for calvarial defect repair, to the best of our knowledge, none of these works have studied the influence of pH, osmolality and hydrogen evolution on the brain tissue. Few in-vitro and in-vivo studies have reported that Mg ions increases the overall number of neural cells as well as differentiation of neural cell to neurons instead of glial cell. However, the cytotoxic effects of excessive Mg ions and associated corrosion products from Mg-based biomaterials, particularly their effects on brain, is poorly understood.

[0179] To assess the systemic toxicity of the FWJ11-CBG, biochemical analysis of the harvested blood and serum was conducted (Tables 6-8) along with the histopathological analysis of the major vital organs responsible for the removal of degradation products arising from the WJ11 alloy degradation were conducted and compared with PEEK-CBG and sham control. The red blood cell, platelet, and white blood cell counts do not reflect any specific trend with implantation time and these counts are not elevated known to occur during post-surgery wound healing or in case of underlying infection. These observations support the evidence of presence of no clinical signs of any surgical site infection. The average values of serum metabolic parameter for liver and kidney functions (Table 7) are within the values of the sham control range. The normal values observed clearly imply that these organs were not affected by the slow degradation of the FWJ11-CBG over time and thus confirms the general safety of the biodegrading magnesium alloy graft. The consistent electrolyte levels including Mg (Table 8) as measured in blood signify that the degradation of FWJ11 did not cause any disturbances in the balance of physiological electrolyte levels. These observations are consistent with other studies where blood biochemistry as well as liver and kidney function were not affected by Mg alloy degradation when implanted in bone, confirming the general safety of the Mg alloying elements. H&E staining of liver, kidney and spleen (Supplementary Tables 4-7) did not reveal any signs of organ alteration or damage further validating the systemic biocompatibility of the WJ11 alloy and its alloying elements. Kidney tissue did not show any focal mineralization, acute inflammatory cell infiltration, or necrosis. Some changes in the kidney tissue were observed for both implant groups and most likely specific to the subject rather than the degradation of the FWJ11-CBG. No

aggregates of inflammatory cells or features of hepatocellular necrosis, such as irregular patchy areas of coagulation necrosis were observed in the liver. Similar to the kidney, any changes noted in the liver tissue was not specific only to the FWJ11-CBG, but instead likely due to the preexisting conditions of the implanted subject. None of the spleen samples showed any significant lesions at any time point for both the implant groups. These observations are not very surprising as both Mg and Sr can be metabolized by the body. Therefore, the very slow degradation and mass loss (maximum 0.18 mg/day and minimum 0.014 mg/day among all the time points, respectively) of the FWJ11-CBG with time should not lead to any toxicity or elemental accumulation in the organs. Most of the previous studies did not show any systemic accumulation of rare earth elements such as yttrium, however, some recent studies reported accumulation of rare earth elements in vital organs mainly in spleen and lymph node from rare earth rich and rapid degrading magnesium alloys. There was no ICP analysis conducted on the digested organ considering the very low mass of the yttrium that will come out of the degraded FWJ11-CBG. Based on degradation results of all the FWJ11 scaffolds after 6 months, the maximum and minimum amount of yttrium was determined to be 210 μg and 26 μg , respectively. Some part of this yttrium will be metabolized and cleared by the body and most likely a small fraction of it would have deposited in various organs and muscles at levels well below 50 parts per billion. This concentration is not high enough for reliably detecting yttrium by our current ICP-OES capability.

[0180] In addition to evaluating the biosafety of the FWJ11-CBG, another major objective of this work was to assess the bone regeneration ability of the graft in an 8 mm critical sized calvarial bone defect with respect to nondegradable and bioinert PEEK. A circular defect of 8 mm diameter is considered to be the smallest size in which the bone cannot completely heal inherently without the presence of other guided material. The volume of new bone formed in the defect site increases with the duration of the implantation time. $\sim 16.8\%$ ($\pm 5\%$) of the defect was filled by new bone in the case of FWJ11 compared to 13.8% ($\pm 2.4\%$) of PEEK after 6 months of implantation. The new bone formation in the defect site was further confirmed by the histological evaluation. These results indicate that the bone regeneration abilities of the FWJ11 and PEEK scaffolds are comparable. Other groups have also evaluated the bone regeneration efficacy of polymer-AZ31 alloy, hydroxyapatite coated pure Mg, and bare and plasma electrolytic oxidation (PEO) based surface modified pure Mg in rat 8.0 mm critical sized calvarial defect. The μCT analysis also clearly indicates that most of the bone formation occurs at the implant surface in contact with the dura. This observation has also been reported by other researchers. Moreover, the histopathological analyses convey the absence of any cartilage and chondrocytes in any of the stained sections and the picrosirius staining clearly indicates the formation of type 1 collagen. These results possibly indicate that the bone formation on these calvarial defects is via intramembranous ossification.

[0181] Magnesium ions are known to stimulate osteogenesis by promoting calcitonin gene-related polypeptide- α (CGRP) and plays a therapeutic role in fracture healing. However, based on bone regeneration results of this study alone, the stimulatory effect of Mg ions in a rat calvarial critical sized defect cannot be confirmed although the results

clearly demonstrate the osteoconductivity of the FWJ11 graft. The published literatures on bone regeneration of critical sized calvarial defects also supports the observations reported in this work. This possible lack of bone stimulating effects or enhanced osteogenesis of the FWJ11-CBG can be due to the slow in-vivo degradation of the implant over time which may not have created localized high alkaline pH that promotes the rapid accumulation of calcium phosphate and new bone formation around the implants. Moreover, the role of high concentration of magnesium ions at the initial inflammation stage is more important than the subsequent active bone repair stage. Recent in vivo and in vitro results have provided compelling evidence showing the cellular activities of macrophages, especially the cytokine release profiles, to be significantly affected by the presence of magnesium ions. A published study also demonstrated that high levels of Mg^{2+} at the beginning of the osteogenic induction of mesenchymal stem cells (MSC) could promote extracellular matrix formation and corresponding mineralization, however, the supplementation of excess magnesium ions at the late stage dramatically impaired the mineralization of MSC. On the other hand, rapid degradation of Mg alloy-based grafts results in the presence of excessive magnesium ions and hydrogen gas bubbles, which can prevent bone formation as has been discussed earlier. The diverse and multifaceted roles of Mg^{2+} ions in bone healing have been investigated mostly in long bone fracture healing model and sub-critical sized bone healing models. The bone formation and bone remodeling stages for calvarial bone and long bones are different and utilize different ossification modes that directly influence the healing patterns and involved cellular and molecular mechanism. Majority of the cranial bone regeneration and repair utilizes intramembranous ossification wherein the dura mater, pericranium, and sutures on the surrounding tissues play important roles, however, the role of Mg^{2+} ions on the various skull formation stages and regeneration is barely known.

[0182] A calvarial defect is generally considered as a non-load bearing defect site and high mechanical compressive and tensile properties of calvarial bone graft is often not necessary. However, grafts possessing similar mechanical properties to the surrounding bone is always preferred for rapid osseointegration and bone regeneration. Measurements of the tensile properties of human calvarial cortical bone revealed that the elastic modulus varies between 5.2 to 21 GPa, failure stress varies from 20.3 to 124.6 MPa and failure strain (%) is in the range of 0.34 to 1.09. The tensile properties of the as extruded WJ11 alloy as shown in Table 5, therefore, matches well with the requirement for the human calvarial bone. In addition to the initial mechanical requirement of the graft, it is also important to evaluate the functional mechanical integration of the FWJ11-CBG and PEEK-CBG into the host calvaria. This was performed by a modified push-out test procedure and the results show that the peak-stress and peak interfacial shear strength for the grafts are not significantly different (FIG. 14). The average peak load (N) for FWJ11-CBG and PEEK-CBG was to be 34.55 N (± 9.1 N) and 30.13 N (± 4.6 N), respectively. This is not surprising as the volumes of bone formation for these grafts are very similar and therefore their integration with the surrounding native bones are also comparable. These peak load values are in the ranges reported for other bone polymer or ceramic bone grafts having similar amount of bone formation in the defect. Lack of any other reports on

Mg alloy based calvarial graft studies prevented us from validating our results further. However, the push-out test clearly indicated that the degradation of the FWJ11 over time does not affect the functional mechanical integration of the degrading graft over time. Finally, it has to be suggested that as a bone biomaterial, the degradation rate of Mg and its alloys should be 0.5 mm per year (mmpy) to provide an effective treatment period of at least 12 weeks. The observed in-vivo corrosion rates of FWJ11-CBG are always well below 0.5 mmpy and therefore it can be concluded that the degrading graft will provide enough mechanical stability during the bone healing period.

[0183] Despite the success of this work in establishing the safety and efficacy of FWJ11 as calvarial bone graft, few limitations of this study are worth specifically noting and considering. First, as seen from the μ CT results, some of the grafts were moved from the defect site as the grafts were not anchored to the surrounding native bone. This raises the possibility that the FWJ11-CBG grafts may have encountered some micro-motion or micro-movement due to pulsating brain as well as due to the normal movement activity of the animal especially during the first few weeks of post-surgery. Micro-motion often leads to poor osseointegration and can result in the formation of a fibrous capsule forms immediately around the implant instead of a direct structural and functional connection between host bone and implant. This may be one of the causes for suboptimal bone formation, variations in the amount of new bone formation and degradation among the grafts at a certain time points. However, the lack of complete bone bridging of the defect site can also be due to the large 8 mm diameter of the defect which is considered as a most aggressive size for a rat. The micro-motion of the FWJ11-CBG and PEEK-CBG may have also have caused minor trauma/injury to the underneath dura mater and brain which was observed in the brain histology of few animals (Supplementary Tables 5, 7). Future study should be conducted using grafts which can be fixed to the surrounding native bone to avoid any micro-motion. In addition to micro-motion of the FWJ11 grafts, the local compositional inhomogeneity of the alloying elements and variations in surface morphology of the implants as observed by SEM may have contributed to the in-vivo degradation. Further heat treatment (e.g., T4 solution treatment) and electropolishing of the as fabricated grafts can eliminate these issues. Second, although the in-vivo degradation of the FWJ11 did not elicit any local and systemic toxicities up to 26 weeks, future study should evaluate the toxicities of the alloying elements after complete degradation of the implant. Third, the amount of newly formed bone, remodeling of the newly formed bone over time, the diverse roles of alloying elements in bone healing, and biomechanical properties of the implant site after complete degradation of the FWJ11-CBG should be carefully evaluated to further assess the clinical feasibility of this technology.

Conclusion

[0184] In the present study we assessed the efficacy and biocompatibility of a fluoride coated magnesium alloy based degradable calvarial scaffold (FWJ11) and the obtained results were compared with a clinically used craniofacial nondegradable biopolymer graft, poly-ether-etherketone (PEEK). The FWJ11 scaffolds demonstrated absence of any apparent local and systemic toxicities up to 26 weeks post implantation and appears to be safe and comparable to

PEEK scaffolds. No hydrogen gas pocket formation was observed or detected in any of the FWJ11 scaffolds containing rats at any time points. The FWJ11 scaffolds demonstrated formation of new bone in the critical sized defect and the amount of new bone formation increases with the duration of the implantation time. However, the volume of new bone formation after a particular implantation time in both the FWJ11 and PEEK scaffolds are not significantly different. Most of the scaffolds from both group of grafts collected at 26 weeks' time point show bony bridging over the partial length of defect. The results also indicate that the bone formation on these calvarial defects is via intramembranous ossification. The FWJ11 scaffolds begin to degrade with time and the degradation rate at various time points is well below the recommended rate of degradation for ideal resorbable bone graft. The peak-stress and peak interfacial shear strength values obtained from the push-out test for both the degradable and nondegradable grafts are not significantly different and therefore, demonstrate that the degradation of the FWJ11 has barely any effect on the overall biomechanical performance of the calvarial graft. Taking altogether, these results suggest that this novel WJ11 alloy calvarial graft has potential in the regeneration of bone in critical sized defects without eliciting any common long-term clinical problems associated with mechanically strong but nondegradable or mechanically weak but rapidly degradable bone grafts.

In the claims:

1. An orthopedic or craniofacial bone fixation component composed of an extruded biodegradable, magnesium alloy, consisting of:

from 0.5 weight percent to 4.0 weight percent of yttrium;
from greater than zero to 1.0 weight percent of zirconium;
from greater than zero to 6.0 weight percent of strontium;
and

a balance of magnesium and impurities due to production, based on total weight of the alloy,

wherein the orthopedic or craniofacial bone fixation component is selected from the group consisting of Kirschner wire, cerclage wire, intramedullary rod, and intramedullary pin.

2. The bone fixation component of claim 1, wherein the obtaining alloying elements comprises one or more alloy elements selected from the group consisting of:

from about 1.0 weight percent to about 9.0 weight percent aluminum;

from about 0.1 weight percent to about 1.0 weight percent of manganese;

from about 0.25 weight percent to about 1.0 weight percent of silver;

and from about 0.1 weight percent to about 1.0 weight percent of cerium.

3. The bone fixation component of claim 1, wherein the magnesium alloy comprises fluoride.

4. A method of preparing an orthopedic or craniofacial bone fixation component, comprising:

preparing a biodegradable, magnesium alloy, consisting of:

from 0.5 weight percent to 4.0 weight percent of yttrium;

from greater than zero to 1.0 weight percent of calcium;

from 1.0 weight percent to 6.0 weight percent of zinc;

from greater than zero to 1.0 weight percent of zirconium;

from 0.10 weight percent to 6.0 weight percent of strontium; and

a balance of magnesium and impurities due to production, based on total weight of the magnesium alloy;

heating the metal alloy to form a molten magnesium alloy;

casting the molten metal alloy to form a cast magnesium alloy; and

extruding the cast metal alloy to form an extruded magnesium alloy,

wherein the orthopedic or craniofacial bone fixation component is selected from the group consisting of Kirschner wire, cerclage wire, intramedullary rod, and intramedullary pin.

5. The method of claim 4, wherein the magnesium alloy further comprises one or more alloy elements selected from the group consisting of:

from about 1.0 weight percent to about 9.0 weight percent aluminum;

from about 0.1 weight percent to about 1.0 weight percent of manganese;

from about 0.25 weight percent to about 1.0 weight percent of silver;

and from about 0.1 weight percent to about 1.0 weight percent of cerium.

6. The method of claim 4, wherein the casting is selected from the group consisting of sand casting, gravity casting, permanent mold casting, direct chill casting, centrifugal casting, low/high pressure die casting, squeeze casting, continuous casting, vacuum casting, plaster casting, lost foam casting, investment casting, and lost wax casting.

7. The method of claim 4, wherein the extruding comprises hot extruding conducted at an extrusion ratio selected from 30:1 and 10:1.

8. The method of claim 4, further comprising homogenizing the cast magnesium alloy.

9. The method of claim 4, further comprising forming a fluoride coating on a surface of the extruded magnesium alloy.

10. A method of preparing an orthopedic or craniofacial bone fixation component, comprising:

preparing a biodegradable, magnesium alloy, consisting of:

from 1.0 weight percent to 6.0 weight percent of zinc;

from greater than zero to 1.0 weight percent of zirconium; and

a balance of magnesium and impurities due to production, based on total weight of the alloy;

heating the metal alloy to form a molten magnesium alloy;

casting the molten metal alloy to form a cast magnesium alloy; and

extruding the cast metal alloy to form an extruded magnesium alloy,

wherein the orthopedic or craniofacial bone fixation component is selected from the group consisting of Kirschner wire, cerclage wire, intramedullary rod and intramedullary pin.

11. The method of claim 10, wherein the magnesium alloy further comprises one or more alloy elements selected from the group consisting of:

from greater than zero to 6.0 weight percent of strontium;

from about 1.0 weight percent to about 9.0 weight percent aluminum;

from about 0.1 weight percent to about 1.0 weight percent of manganese;

from about 0.25 weight percent to about 1.0 weight percent of silver; and

from about 0.1 weight percent to about 1.0 weight percent of cerium.

12. The method of claim 10, wherein the casting is selected from the group consisting of sand casting, gravity casting, permanent mold casting, direct chill casting, centrifugal casting, low/high pressure die casting, squeeze casting, continuous casting, vacuum casting, plaster casting, lost foam casting, investment casting, and lost wax casting.

13. The method of claim 10, wherein the extruding comprises hot extruding conducted at an extrusion ratio selected from 30:1 and 10:1.

14. The method of claim 10, further comprising homogenizing the cast magnesium alloy.

15. The method of claim 10, further comprising forming a fluoride coating on a surface of the extruded magnesium alloy.

16. A method of preparing an orthopedic or craniofacial bone fixation component, comprising:

preparing a biodegradable, magnesium alloy, consisting of:

from 0.5 weight percent to 4.0 weight percent of yttrium;

from greater than zero to 1.0 weight percent of zirconium;

from greater than zero to 6.0 weight percent of strontium; and

a balance of magnesium and impurities due to production, based on total weight of the alloy;

heating the metal alloy to form a molten magnesium alloy;

casting the molten metal alloy to form a cast magnesium alloy; and

extruding the cast metal alloy to form an extruded magnesium alloy,

wherein the orthopedic or craniofacial bone fixation component is selected from the group consisting of Kirschner wire, cerclage wire, intramedullary rod, and intramedullary pin.

17. The method of claim 16, wherein the magnesium alloy further comprises one or more alloy elements selected from the group consisting of:

from about 1.0 weight percent to about 9.0 weight percent aluminum;

from about 0.1 weight percent to about 1.0 weight percent of manganese;

from about 0.25 weight percent to about 1.0 weight percent of silver;

and from about 0.1 weight percent to about 1.0 weight percent of cerium.

18. The method of claim 16, wherein the casting is selected from the group consisting of sand casting, gravity casting, permanent mold casting, direct chill casting, centrifugal casting, low/high pressure die casting, squeeze cast-

ing, continuous casting, vacuum casting, plaster casting, lost foam casting, investment casting, and lost wax casting.

19. The method of claim **16**, wherein the extruding comprises hot extruding conducted at an extrusion ratio selected from 30:1 and 10:1.

20. The method of claim **16**, further comprising homogenizing the cast magnesium alloy.

21. The method of claim **4**, further comprising forming a fluoride coating on a surface of the extruded magnesium alloy.

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